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The identification and characterization of protein tyrosine phosphatases expressed in the developing rat brain

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The Identification and Characterization of Protein Tyrosine Phosphatases Expressed in the Developing Rat Brain

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ABSTRACT

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Previous studies of the developing nervous system have shown that cell-cell and cell-matrix interactions are involved in a variety of processes such as the proliferation, migration and differentiation of neurons. While many cell surface molecules have been identified, the signal transduction mechanisms through which they alter cellular responses are poorly understood. Recent studies have described a new and large family of enzymes, protein tyrosine phosphatases (PTPases), that may play a key role in transduction of cell surface events. Opposing the actions of protein tyrosine kinases (PTKs), PTPases may determine the state of tyrosine phosphorylation of a protein and regulate its function. Within the family of PTPases, two subgroups have been characterized: low molecular weight cytoplasmic (non-receptor) PTPases and high molecular weight transmembrane (receptor) PTPases. Receptor PTPases have fibronectin type III and/or immunoglobulin-like domains in their extracellular domains, suggesting that they may have dual functions: cell adhesion and signal transduction. Such molecules may play a role in cellular recognition events that mediate the accurate assembly of the nervous system.

Using PCR with degenerate primers and a neonatal rat cortex cDNA library, we have identified seven PTPases expressed in the developing rat brain. Four of these are transmembrane PTPases: LAR, LRP, RPTPγ and

CPTP1. Three are non-receptor PTPases: PTP-1, P19-PTP and SHP. Within the embryonic and early postnatal brain, the seven PTPases have overlapping, yet unique distributions. The expression of each of these genes are regulated in a temporally and spatially restricted pattern. Two PTPase genes, CPTP1 and P19-PTP, are expressed in a developmentally-regulated pattern. These two PTPases are expressed at their highest levels during neurogenesis and neuronal differentiation and are markedly down-regulated during postnatal life. Both CPTP1 and P19-PTP are widely distributed in the developing brain. In contrast, a non-receptor protein tyrosine phosphatase, PTPH1, is found in a region-specific pattern. PTPH1 is expressed in all adult thalamic nuclei generated by the dorsal thalamus and is absent in the nuclei generated from the ventral thalamus.

The abundance and differential regulation of PTPase genes expressed in the developing brain suggest that they play a role in cell-cell interactions mediating neural differentiation. The comparative analysis of the expression patterns presented in this dissertation can guide further biochemical and genetic experiments that would allow a better understanding of their precise cellular function.

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GENERAL INTRODUCTION

The mammalian central nervous system (CNS), more than any other tissue, contains many distinct regions, each containing a large number of different cell types with highly specialized functions. One of the greatest challenges in neurobiology today is to understand how the regional and cellular diversity of the adult CNS is generated from a neural tube composed of an morphologically homogeneous group of progenitor cells. Recent evidence suggest that cellular interactions during the proliferation, migration and differentiation of cells in the developing nervous system play a role in the generation of cellular diversity. Such interactions start with the neuronal progenitor cells that are located in spatially restricted zones in the embryonic brain. These progenitors first undergo a phase of proliferation during which the rate of proliferation and identity of the progeny are regulated by cell-cell interactions (Gao et al., 1991; McConnell and Kaznowski, 1991). Subsequently, post-mitotic neurons migrate to their final position in the neuraxis and extend neurites. Both migration and axon elongation are mediated by interactions with adjacent cells and matrix (Rakic et al., 1994; Kapfhammer and Schwab, 1992). Finally, the mature regional identity of neurons is established; because the patterns of connectivity and molecular phenotype can be altered by transplanting areas of the brain to ectopic locations, these mature features are also determined, at least in part, by intercellular cues (O'Leary and Stanfield, 1989; Schlaggar and O'Leary, 1991; Barbe and Levitt, 1991). While the determination of neuronal phenotype by cell-cell interactions throughout development is well established, the molecular mechanisms involved in these processes are largely unknown.

Cell-cell interactions and their effects on cellular phenotype are mediated

by cell surface molecules and associated second messenger systems. While the cell surface molecules have been thought of previously as "cell adhesion molecules", the identification and characterization of increasing numbers of such molecules has made it increasingly clear that the function they perform is not merely a matter of extracellular "stickiness", but is a complex phenomenon involving transmembrane signaling and cytoplasmic responses. The significance of cell surface proteins in signal transduction has been demonstrated most clearly in the immune system. For example, in the interaction between leukocytes and endothelium, initial adhesion leads to "activation" of the leukocytes which is mediated by second messengers (Springer, 1990; Butcher, 1991). Similarly, the binding of helper T cells to antigen presenting cells is followed by a cytoskeletal reorganization that is regulated by phosphorylation (Kupfer and Singer, 1989). In the nervous system, as well, many cellular processes are regulated by protein phosphorylation, balanced by the competing activities of protein kinases and phosphatases. In fact, genetic studies in Drosophila illustrate that cell adhesion and protein phosphorylation can reflect two components of a single process (Elkins et al., 1990).

In order to understand the molecular basis of cellular interactions during neural development, it is necessary to identify and characterize the proteins that mediate cell surface recognition and that transduce signals from the cell surface to the cytoplasm. One family of proteins that can fulfill both functions is the receptor protein tyrosine phosphatases (PTPases). This dissertation explores the identity and the expression patterns of PTPases found in the developing rat brain. In this introduction, first I will briefly review the role of tyrosine phosphorylation in the developing nervous system and then focus on the PTPase family. Chapter 1 describes the PCR-based approach that we used to

identify seven PTPases from newborn rat neocortex and compares their expression patterns. Chapter 2 focuses on two of the seven PTPases identified in the first chapter and characterizes their expression patterns throughout neural development. Chapter 3 examines the expression of a non-receptor PTPase that is particularly enriched in the rat thalamus. Finally, the dissertation concludes with a discussion of each of the PTPases studied in this work and some concluding remarks about future experiments elucidating the role of PTPases in neural development.

TYROSINE PHOSPHORYLATION DURING DEVELOPMENT: THE STORY OF KINASES

Regulation of protein function through tyrosine phosphorylation is critical in the control of many developmental events, including cellular proliferation and differentiation. The state of tyrosine phosphorylation is determined by the opposing actions of protein tyrosine kinases (PTKs) and PTPases. In the last several years, a growing number of PTKs and PTPases have been identified in various species and tissues, including the mammalian CNS. Recently, research from several different areas has converged to begin to elucidate the roles played by PTKs.

First, studies of growth factors and their receptors have markedly advanced our understanding of the functional significance of PTKs. The *trk* family of tyrosine kinases, first identified as genes with oncogenic potential, are now known to function as high affinity neurotrophin receptors (Chao, 1992). In addition, the receptors for other peptide growth factors, such as β-PDGF, basic FGF, EGF and IGF-I, are also transmembrane PTKs and are expressed in neural tissues (Schlessinger and Ullrich, 1992; Chao, 1992). The effects of these growth factors on PC12 cells indicate that they, too, may play a role in

neural development (Heasley and Johnson, 1992). More recently, another group of receptor PTKs, the *neu* oncogene and its homologs, have been identified as the receptors for the neuregulin family of neural differentiation factors, such as ARIA (Peles and Yarden, 1993).

Second, mutations in several PTK genes in Drosophila cause aberrations in neural development (reviewed in Shilo, 1992). Several Drosophila mutants indicate that receptor PTKs are crucial for determining the identity of neuroblasts in the CNS [*faint little ball* (Schejter and Shilo, 1989)], establishing the number and spacing of photoreceptors in the eye imaginal disc [*ellipse* (Baker and Rubin, 1989)], differentiation of the photoreceptors [*sevenless* (Rubin, 1991)], and glial migration [*breathless* (Klambt et al., 1992)]. In addition, a trk-like Drosophila protein, Dtrk, promotes homophilic cell adhesion (Pulido et al., 1992).

Third, non-receptor PTKs (src, fyn, yes) are enriched in growth cones (Maness, 1992; Rudd et al., 1993) suggesting that they play a role in neurite outgrowth. Recent experiments using transgenic mice in which each of these PTKs have been knocked-out strongly suggest that each kinase has a distinct and non-redundant role in mediating axonal growth in response to specific cell adhesion molecules. For example, src is essential for L1-mediated axonal growth while fyn plays a similar role in intracellular signaling initiated by NCAM (Ignelzi et al., 1994; Beggs et al., 1994).

Finally, using a PCR based approach, several groups have identified novel putative PTKs that are expressed preferentially in the developing CNS (Lai and Lemke, 1991; Sanchez et al., 1994). One large family is the eph family of transmembrane PTKs some members of which are expressed in a segmental pattern in the hindbrain rhombomeres (Nieto et al., 1992; Henkemeyer et al., 1994; Becker et al., 1994). Until recently, this family of PTKs were orphan

receptors. However, a family of transmembrane molecules are now identified as ligands for eph kinases (Cheng and Flanagan, 1994; Beckmann et al., 1994). In summary, there is ample evidence that PTKs play an important role in neural development. In contrast, much less is known about the PTPases.

PTPase FAMILY

The hallmark of the PTPase family is the presence of a conserved catalytic domain that preferentially dephosphorylates proteins on tyrosine residues. The catalytic domain was first identified because of sequence homology between the first biochemically purified cytoplasmic PTPase (PTP-1B) and a lymphocyte transmembrane protein of previously unknown function, CD45 (Charbonneau et al., 1989). CD45 was subsequently shown to possess PTPase activity (Tonks et al., 1988), and this activity was associated with a 230 amino acid catalytic domain (Fischer et al., 1991). This then led to a search for other PTPases by PCR and low stringency hybridization. The PTPases identified so far fall into two major groups depending on whether or not they have a membrane spanning region. The **non-receptor (cytoplasmic) PTPases** are small molecules with only a single catalytic region and lacking a transmembrane domain. In contrast, the **receptor (transmembrane) PTPases** are larger molecules with a large extracellular domain, a single transmembrane domain and, in almost all cases, two tandem catalytic domains in the intracellular, C-terminal region of the protein. Two exceptions, human PTPβ and Drosophila DPTP10D, have only one catalytic domain. Deletional and mutational analyses indicate that most of the catalytic activity is associated with the first (membrane-proximal) domain (Streuli et al., 1990; Desai et al., 1994). The second (C-terminal) domain may have some catalytic (Tan et al., 1993) and/or regulatory activities (Streuli et al., 1990). In contrast to the very

high degree of homology in the intracellular domains of the receptor PTPases, the extracellular regions are strikingly divergent in size and structure. The extracellular domains of some receptor PTPases resemble cell adhesion molecules, containing immunoglobulin (Ig)-like and fibronectin type III (FN III)-like repeats. In fact, like other proteins with Ig- and FN III-like domains, two receptor PTPases, PTPμ and PTPκ, demonstrate homophilic binding activity (Brady-Kalnay et al., 1993; Gebbink et al., 1993; Sap et al., 1994). It is therefore tempting to speculate that receptor PTPases play dual roles, as cell adhesion and signal transduction molecules.

Since phosphorylation is a reversible process mediated by both the addition and removal of phosphate groups, it is likely that PTPases, just like PTKs, will have significant functions in the developing CNS. Given the possible function of PTPases in determination of cellular phenotype and our lab's interest in the generation of cellular diversity in the mammalian CNS (Hockfield and McKay, 1985; Hockfield and Sur, 1990; Martin et al., 1992), I have examined the expression of PTPases in the rat CNS during development. The results of this work are presented in the next three chapters and are discussed within the framework of the explosive progress that has occurred in the PTPase field in the last six years.

CHAPTER 1

SEVEN PROTEIN TYROSINE PHOSPHATASES ARE DIFFERENTIALLY EXPRESSED IN THE DEVELOPING RAT BRAIN

SUMMARY

Regulation of protein function through tyrosine phosphorylation is critical in the control of many developmental processes such as cellular proliferation and differentiation. Growing evidence suggests that tyrosine phosphorylation may also regulate key events in neural development. While a large body of data has demonstrated that protein tyrosine kinases play an important role in neural development, much less is known about their counterparts, protein tyrosine phosphatases (PTPases). Using PCR with degenerate primers and a neonatal rat cortex cDNA library, we have identified seven PTPases expressed in the developing rat brain. Four of these are transmembrane PTPases: LAR, LRP, RPTPγ and CPTP1. Three are non-receptor PTPases: PTP-1, P19-PTP and SHP. Northern hybridization analysis demonstrates that only CPTP1 is preferentially expressed in neural tissues while the others are found abundantly in non-neural tissues as well as in the brain.

Within the embryonic and early postnatal brain, the seven PTPases have overlapping, yet unique distributions. For example, LAR mRNA is highly expressed by both proliferating and post-mitotic cells in the cerebral cortex at embryonic day 17 and in all layers of the cortex at postnatal day 4. In contrast, RPTPγ mRNA is expressed by post-mitotic neurons in the embryo and predominantly by neurons in the superficial layers of the postnatal cortex.

Several of the PTPases examined here are expressed at very high levels in the embryonic cortical plate and postnatal neocortex, including the subplate

and subventricular zone. The spatial and temporal regulation of PTPase gene expression suggest that these PTPases have important roles in signal transduction during early neuronal differentiation and neural development.

INTRODUCTION

One of the most ubiquitous intracellular signaling systems is phosphorylation of proteins on serine, threonine and tyrosine residues. Regulation of protein function through tyrosine phosphorylation is known to be critical in the control of many developmental processes, including cellular proliferation and differentiation. Growing evidence suggests that tyrosine phosphorylation and dephosphorylation may also play key roles in neural development (Wagner et al., 1991). The opposing actions of protein tyrosine kinases (PTKs) and protein tyrosine phosphatases (PTPases) determine the state of protein tyrosine phosphorylation. A growing number of PTKs and PTPases have been identified in various species and tissues, including the mammalian central nervous system (CNS).

While new members of the PTPase family are being cloned and sequenced at a rapid pace, the physiological roles played by these newly identified enzymes remain largely unknown. One possibility is that PTPases act as negative regulators and directly oppose the actions of protein tyrosine kinases (PTKs) (Brown-Shimer et al., 1992). However, they can also take part in the same pathway as PTKs and work in concert them. For instance, the lck and fyn tyrosine kinases are activated when they are dephosphorylated by CD45 (Koretzky, 1993). Another receptor PTPase, LRP, can similarly regulate the activity of src kinase (Zheng et al., 1992). Furthermore, receptor PTKs can associate with and activate non-receptor PTPases by phosphorylation, as seen in the case of syp (Vogel et al., 1993). Given the abundance, diversity and significance of PTK expression in the developing CNS (Lai and Lemke, 1991), it is tempting to postulate that the involvement of the PTPases will prove to be crucial as well.

Growing evidence suggests that PTPases play key roles in neural differentiation and axonal outgrowth. NGF-induced neuronal differentiation of PC12 cells, for instance, is associated with a dose-dependent increase in PTPase activity (Aparicio et al., 1992). In addition, the expression of a receptor PTPase (LRP) is up-regulated during neuronal differentiation of embryonal carcinoma and neuroblastoma cell lines. Overexpression of LRP in an embryonal carcinoma cell line results in a higher fraction of cells undergoing neuronal differentiation (Den Hertog et al., 1993). Finally, three receptor PTPases are differentially expressed on subsets of developing axons in Drosophila. Following the completion of axonogenesis, the mRNAs for all three PTPases are down-regulated (Yang et al., 1991; Tian et al., 1991; Hariharan et al., 1991). Consistent with a role for PTPases in mammalian neural development, the overall level of tyrosine phosphorylation indicates that PTPases are particularly active in the late embryonic brain (Maher, 1991).

Because of our interest in the generation of cellular diversity and connectional specificity in the developing nervous system, we examined the expression of PTPases in the neonatal rat CNS. We have reported the identification of two PTPases that are expressed at high levels in the neonatal rat cortex and thus are named Cortex-enriched Protein Tyrosine Phosphatases (CPTPs) (Sahin and Hockfield, 1993; see chapter 2). This chapter describes the indentification of five other PTPases that are found in the neonatal rat neocortex. Although these five PTPases were previously cloned from other tissues, their expression in the developing cortex had not been examined. Here we used northern hybridization to determine whether any of the seven PTPases is expressed in a neural-enriched and/or developmentally-regulated pattern. We also performed a detailed comparative in situ hybridization analysis of expression patterns of all seven PTPases in the pre- and early post-natal brain.

Our analysis shows that some PTPases are down-regulated over the course of cortical development while others are up-regulated. Differential spatial and temporal expression of the seven PTPases suggest that they play distinct roles in various stages of neural development.

MATERIALS AND METHODS

RNA extraction and cDNA library synthesis

Total cellular RNA was extracted from occipital cerebral cortex of postnatal day zero (P0) Sprague-Dawley rats using the guanidine thiocyanate/cesium chloride ultracentrifugation method (Bothwell et al., 1990). Poly A^+ RNA was isolated by one pass through an oligo dT cellulose (type III, Collaborative Research) affinity column (Sambrook et al., 1989). Oligo dT primed cDNA synthesis was carried out with the Superscript Plasmid kit (GIBCO/BRL). Briefly, the kit uses a *Not* I primer-adapter and $RNaseH^-$ M-MLV reverse transcriptase for the first strand and *E. coli* RNase H, DNA pol I and DNA ligase for the second strand synthesis. Double stranded cDNA was blunt-ended with T4 DNA pol, cut with *Not* I and size fractionated by column chromatography. cDNAs larger than 500 base pairs (bp) were ligated directionally into a modified Bluescript vector, E61, gift of J.L.R. Rubenstein (Rubenstein et al., 1991). The cDNA library contained $3.2x10^6$ clones with an average insert size of 900 bp.

PCR Amplification

The cDNA library was used as a template for amplification using *Taq* polymerase (GeneAmp, Perkin Elmer) and degenerate primers (see Figure 1 for position and sequence of primers). Twenty five pmol each of sense and antisense primers were used in 25 µl reactions in *Taq* buffer (10 mM Tris-HCl

pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% gelatin) with 0.6 units of *Taq* polymerase, 200 µM dNTPs and 25 ng of cDNA library as template. PCR was carried out in a Perkin Elmer DNA Thermal Cycler for 35 cycles. The first five cycles of PCR included a 30 sec denaturation at 94°C, 1 min annealing at 37°C, a slow ramp (1° every 4 sec) to 72° and 3 min extension at 72°C. The next 30 cycles ran as follows: 94° for 30 sec, 50° for 1 min and 72° for 2 min. Amplified PCR fragments were then ligated without size selection into the pCRII vector (Invitrogen). Since our initial low stringency PCR screen yielded many clones that were not homologous to PTPases (Sahin and Hockfield, 1993), we utilized an additional re-screening step using a conserved internal primer. This internal primer was based on the amino acid sequence KC(D/A)(K/E/Q)YWP and had the following oligonucleotide sequence: 5'-AA(A/G)TG(C/T)G(A/C)(C/T)(A/C/G)A(A/G)TA(C/T)TGGCC-3'. PCR was performed with the internal primer and the original downstream primer for 30 cycles (of 94° for 30 sec, 50° for 1 min and 72° for 2 min each). The transformants that were positive by this re-screen were then sequenced by the dideoxy chain termination method (Sequenase, U.S. Biochemical) on both strands using M13 reverse and T7 primers. Sequence analyses were conducted using GCG software (Genetics Computer Group, 1991). The PCR clones were then used for the northern and in situ hybridization analyses as described below.

Northern hybridization

Total cellular RNA was extracted from various tissues of Sprague-Dawley rats using the guanidine thiocyanate/cesium chloride ultracentrifugation method (Bothwell et al., 1990). For the analysis of developmental regulation, the cerebral cortex was dissected from animals at embryonic day 16 (E16),

postnatal day 0 (P0), P4, P14, P30 and adult, using care to exclude the underlying diencephalon and the hippocampus. Total RNA was also extracted from P35 liver as a non-neural control. Twenty five µg of total RNA from each sample was resolved by electrophoresis on a 1% agarose gel containing 2.2 M formaldehyde and 1X MOPS buffer, and transferred to Zeta-probe (Bio-Rad) by capillary blotting. Blots were pre-hybridized in 7% SDS, 1% BSA, 0.5 M phosphate buffer pH 6.8 (PB), 1 mM EDTA for 1 hour at 65°C. Hybridization was carried out in the same solution for 18 hours at 65°C. Hybridization solution contained 1.2 x 10^6 cpm/ml of probe made by random primed labeling. After hybridization, the blot was washed twice in 5% SDS, 0.5% BSA, 40 mM PB, 1 mM EDTA and twice in 1% SDS, 40 mM PB, 1 mM EDTA at 65°C for 20 minutes each. RNA molecular weight standards (GIBCO/BRL) were also run on the blots to estimate the sizes of the transcripts. Cyclophilin, which is expressed at a constant relative abundance throughout brain development, was used as a control for equal loading of lanes (Lenoir et al., 1986).

For the analysis of tissue distribution, northern blots containing RNA from various tissues of adult Sprague-Dawley rats and several cell lines were generously supplied by Dr. Eric Bellefroid, Yale University. Extraction of RNA and northern blotting were performed as described above. Each lane of the blots contained 20 µg of total RNA. Equality of loading levels was confirmed by adding ethidium bromide to each RNA sample and visualizing the 28S and 18S rRNAs under UV illumination. The blots were then hybridized and washed as described above and exposed to Amersham Hyperfilm between two intensifying screens for 1-7 days.

In situ hybridization

In situ hybridization was performed as in Martin et al. (1992) with

modifications. Twelve micron frozen sections were thaw-mounted onto gelatin coated slides and post-fixed in 0.1 M sodium phosphate buffered 4% paraformaldehyde (pH 7.4). Sections were rinsed in 1X PBS (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO4), 2X SSC and acetylated with 0.5% acetic anhydride in 0.1 M triethanolamine (pH 8.0). Sections were then rinsed in 2X SSC, 1X PBS, dehydrated in ethanols and delipidated in chloroform. Sections were prehybridized in 2X SSC, 50% formamide at 50°C for 1 hr, and then hybridized in 50% formamide, 1X Denhardt's, 0.75 M NaCl, 10% dextran sulfate, 30 mM DTT, 10 mM Tris-HCl pH 7.5, 1mM EDTA, 0.5 mg/ml tRNA, 100 µg/ml salmon sperm DNA and 1 $x10^6$ cpm probe at 50°C for 12-15 hours. The [^{35}S]-CTP (NEN) labeled cRNA probes were generated using the T7 or SP6 promoters in the TA vector and the Riboprobe system (Promega). Following hybridization, slides were treated with 20 µg/ml RNase A, washed in 2X SSC, 50% formamide, 0.1% BME at 58°C and in 0.1X SSC/0.1% BME at 65°C for 30 min each. In order to provide a more accurate comparison of expression patterns, all of the probes were hybridized and washed simultaneously under identical conditions. Slides were then exposed to Amersham Hyperfilm for 1-9 days. Autoradiograms were used as negatives to print the figures. For higher resolution, the slides were dipped into NTB-2 emulsion (Kodak) and developed after 7-28 days. Some slides were counter stained with cresyl violet to visualize cytoarchitectonic details.

RESULTS

PCR from a P0 cortex library resulted in the identification of seven PTPase domains.

To identify PTPases expressed in neonatal rat cortex, PCR amplification

was carried out with primers corresponding to the conserved catalytic domains of previously reported receptor PTPases. Oligonucleotide primers corresponding to amino acid sequences DFWRM(I/V)W (upstream) and HCSAGVG (downstream) were synthesized using most common codon usage tables to reduce degeneracy (Lathe, 1985) (Fig. 1). A postnatal day zero (P0) rat neocortex cDNA library was used as the DNA template for the amplification. The initial PCR performed with low stringency annealing conditions yielded many clones without any homology to PTPase domains (Sahin and Hockfield, 1993). Therefore, a re-screening step using an internal PCR primer was used to eliminate PCR products lacking the highly conserved PTPase sequence. Indeed, only 35 out of 62 original PCR clones were positive with this secondary screen. Subsequent sequence analysis showed that 33 of these 35 encoded PTPase domains.

The 33 clones can be sorted into seven different groups, each corresponding to a previously identified PTPase (Fig. 2). The first four groups (representing 21 of the clones) correspond to either the first or second catalytic domains of four receptor PTPases. The remaining three groups (representing 12 clones) correspond to the single catalytic domain of three non-receptor PTPases.

The first group consists of six clones which are 100% identical to the first catalytic domain and one clone which is identical to the second domain of rat LAR (Leukocyte common Antigen Related protein) (Pot et al., 1991; Yu et al., 1992). The second group consists of seven identical clones with sequences that match the first domain of rat LRP (Leukocyte common antigen Related Phosphatase) (Moriyama et al., 1992). The third group of five clones represents CPTP1, a receptor PTPase we and others have described recently (Sahin and Hockfield, 1993; see chapter 2; Walton et al., 1993; Pan et al., 1993; Yan et al.,

1993). Two clones are identical to the first domain of CPTP1 and three to its second domain. Finally, the fourth group has two identical clones that show 90% nucleotide identity to the first catalytic domain of mouse RPTPγ (Barnea et al., 1993), and 71% identity to human PTPζ (Krueger and Saito, 1992). In their deduced amino acid sequence, these clones are 97% identical to mouse RPTPγ, and thus probably represent the rat homologue of this gene.

The remaining 12 clones comprise three groups, each of which corresponds to a non-receptor PTPase. The eight clones in the first group are identical to PTP-1, originally isolated from the rat hypothalamus (Guan et al., 1990). PTP-1 is the rat homolog of PTP-1B originally identified from human placenta (Charbonneau et al., 1989). The second group contains three clones identical to CPTP2, which we have reported (Sahin and Hockfield, 1993, see chapter 2). Since the full-length sequence of this PTPase has been named P19-PTP (Den Hertog et al., 1992), we will refer to it by this name in the rest of this chapter. Finally, one copy of a PCR sequence was identified with 96% nucleotide identity to mouse SHP (SH2-domain Phosphatase) (also known as PTP1C and HCP; Shen et al., 1991; Matthews et al., 1992; Yi et al., 1992).

Northern hybridization demonstrates that different PTPases have different developmental expression patterns in the cortex.

Representative clones from each group were used to characterize the expression patterns of the seven PTPases. Since the homology among PTPases in the first catalytic domains is usually less than that in the second (Streuli et al., 1990; Sahin and Hockfield, 1993), we used the first catalytic domains as probes in order to minimize the probability of cross-hybridization to other PTPases. We focused on LAR and CPTP1, the PCR clones that showed the highest sequence identity (74%), to confirm the specificity of our probes and

to rule out cross-reactivity. Even at 74% homology, the calculated melting temperature of CPTP1-LAR hybrids is lower than the stringency conditions used for the hybridization. The hybridization patterns of the two probes do not change when the stringency of the hybridization is increased by raising the wash temperature to 70°C. Therefore, even in the case of the two most homologous genes, the PCR clones that we used as probes for northern and in situ hybridization analyses are specific for their respective genes, and the signals that they generate do not represent cross-hybridization.

The expression patterns of the seven PTPases during cortical development were examined using northern blots of RNA isolated from rat cerebral cortex of different ages. This analysis confirmed the PCR results, that all seven PTPases are expressed in the developing cortex (Fig. 3A). In fact, for several receptor PTPases (LAR, RPTPγ, CPTP1) multiple transcripts, each with a distinctive time course of expression, are present in the cortex.

LAR has three major transcripts with approximate sizes of 4.0, 6.0 and 8.0 kb. The ratio of expression of these three transcripts changes during cortical development. In the embryonic brain, the 4.0 kb band is the most abundant. During development, the 8.0 kb transcript is substantially up-regulated, and reaches a peak in the adult cortex, where it is the most abundant form. The 6.0 kb transcript has the lowest intensity at all ages. None of the three bands is readily detectable in RNA from P35 liver.

In contrast to LAR, LRP is encoded by a single transcript in the cortex (Fig. 3A). The mRNA, which is around 3.5 kb, remains at a roughly constant level throughout cortical development. The P35 liver does not contain any LRP message that can be detected by this analysis.

The northern blot analysis for RPTPγ shows two major high molecular weight transcripts, at approximately 7.0 and 9.0 kb, and two minor transcripts at

approximately 4.3 and 3.3 kb (Fig. 3A). The two major transcripts display roughly parallel temporal regulation patterns, both reaching their highest levels in the second postnatal week and then gradually declining to adult levels. The 3.3 kb mRNA is detected at moderate levels at E16 and is then markedly down-regulated postnatally. The 4.3 kb mRNA does not appear to be temporally regulated.

CPTP1 and P19-PTP, whose developmental expression patterns will be described in detail in Chapter 2, are included in Figure 3A for comparison. Briefly, CPTP1 is encoded by two major transcripts around 6.5 and 7.8 kb. The higher molecular weight mRNA predominates in the embryonic cortex and is down-regulated during development. The 6.5 kb message appears only postnatally in the cortex and becomes the more abundant form by around P14 and remains so in the adult cortex. In contrast, the P19-PTP probe recognizes a single transcript around 3.5 kb. The mRNA for this non-receptor PTPase is expressed at highest levels at E16 and is down-regulated to its adult level shortly after birth.

The mRNA species recognized by the PTP-1 probe is approximately 4.5 kb (Fig. 3A). It is detected as early as E16 in the cortex and is slightly down-regulated through postnatal life. In contrast to the other PTPases, SHP mRNA is more abundant in the liver than it is at any age in the cortex. The expression level of the 2.4 kb SHP transcript remains essentially constant during cortical development (Fig. 3A).

PTPases have distinct expression patterns in adult tissues, with CPTP1 highly enriched in the brain.

In order to characterize the tissue distribution of the seven PTPases, we examined their expression in various adult rat tissues and in five rodent cell

lines. This analysis demonstrates that PTPase genes have overlapping yet unique expression patterns. In the adult brain, some of the PTPases are present at high abundance whereas others are barely detectable.

LAR mRNA is detected in many tissues with the highest levels in the lung, followed by brain, thymus, testis and ovary (Fig. 3B). The higher level of expression found in the thymus compared to the spleen is consistent with a higher level of expression by T-cells than by B-cells (Streuli et al., 1988). In addition to the three transcripts found in the cortex, a smaller transcript that runs slightly above 18S rRNA is found in the lung, thymus, testis and myeloid leukemia cell lines. The "brain" lane on the tissue northern shown in Fig. 3B represents total brain RNA, rather than cerebral cortical RNA (as in Fig. 3A). Therefore, the relatively lower level of expression of the 6.0 and 8.0 kb bands compared to the 4.0 kb band is most likely due to the enrichment of the larger RNA species in the cortex compared to the rest of the brain, as recently reported by Longo et al. (1993).

LRP expression is higher in the brain than in the other adult tissues (Fig. 3B). Lung and ovary have intermediate levels while heart and kidney have low levels of LRP mRNA. Leukemia cell lines also express significant levels of LRP.

RPTPγ shows a more complicated tissue distribution (Fig. 3B). The high molecular weight transcripts seen in the cortex are present at equally high levels in the heart, lung, kidney and ovary. A previous study reported only the two high molecular weight transcripts by northern analysis of mouse tissues (Barnea et al., 1993). The long exposures used here to visualize RPTPγ RNAs in muscle and testis also clearly show the 4.3 kb band in several tissues. However, consistent with the previous study of RPTPγ expression (Barnea et al., 1993), the lower molecular weight transcripts are present at dramatically lower abundance, except in the E16 cortex (Fig. 3A). The smallest molecular weight

band found in the cortex appears to be brain specific. An even smaller transcript, running close to 18S rRNA, is found exclusively in the testis.

Initially we examined the expression of CPTP1 and P19-PTP in only two non-neural tissues, liver and kidney (Sahin and Hockfield, 1993). This more extensive tissue analysis confirms our initial interpretations (Fig. 3B): the 6.5 kb transcript of CPTP1 is indeed neural-specific; it is not detected in any other organs or cell lines examined, even after long exposures. The 7.8 kb transcript is also found in the lung, muscle and testis, although at much lower levels than in the brain. Interestingly, the lymphocytic leukemia cell line, NF8D5, and the pituitary cell line, GC, predominantly express the 7.8 kb message. P19-PTP mRNA, as we described previously, is found in the adult brain but is not neural-specific; in fact, its abundance is much greater in the lung than in the brain (Fig. 3B). Lower levels of P19-PTP are also detected in the kidney, testis and several cell lines.

PTP-1 message, which runs slightly below 28S rRNA, is only faintly detected in all lanes of the tissue northern containing 20 μg total RNA (Fig. 3B). This is consistent with the original report of this PTPase, in which detection of PTP-1 mRNA required 5 μg of poly-A^+ RNA (Guan et al., 1990). Even with the faint signals we obtained here, the lung appears to express the greatest amount of PTP-1. This observation supports the results of a recent PCR experiment which identified many more copies of PTP-1 than any other PTPase in the lung epithelia (Rotin et al., 1994). Smaller transcripts of PTP-1 can be detected in the testis and in the fibroblast and myeloid leukemia cell lines.

SHP was originally cloned from a pre-B-cell line (Matthews et al., 1992). The tissue distribution demonstrates highest levels in the lung (as described by Plutzksy et al., 1992), followed by thymus, spleen and two leukemia cell lines. Although we detect a signal in the cortex blot (Fig. 3A), no signal is detectable in

the brain lane of the tissue blot. In fact, no signal is detectable even in the liver, which contains more SHP mRNA than the cortex (Fig. 3A). It appears, therefore, that the adult brain contains very low levels of SHP transcript. Given the high levels of SHP expression by hematopoietic cells, we initially speculated that the low level of expression detected in the cortex may be due to the presence of blood cells in the brain (Sahin et al., 1995). In fact by using an antisera to SHP (supplied by C. Siminovitch), we have detected the SHP protein in microglia and macrophages in the developing and adult brain (data not shown).

PTPases have distinct expression patterns in the embryo.

Sagittal sections of E17 embryos were probed with radiolabeled antisense RNA corresponding to each of the PTPase domains used for northern hybridization. This in situ hybridization analysis gives an overall view of the expression pattern of each of the PTPases. Because each probe recognizes all the transcripts of each PTPase, this analysis cannot, of course, provide information about possible differences in expression of the different transcripts. As a positive control for neuronal RNA expression, near adjacent sections were probed with a clone for the middle subunit of neurofilament (NF) (Lewis and Cowan, 1986) (Fig. 4H). A sense probe for LAR was used as a negative control for non-specific hybridization and showed no hybridization signal (Fig. 4I). At E17, cortical neurons are being generated in the ventricular zone, and newly born cells are migrating to their appropriate locations. Consistent with the northern analysis, many PTPases show high levels of hybridization to the embryonic cerebral cortex at this age.

Within the CNS, LAR is expressed at high levels in the cortex, midbrain, and spinal cord (Fig. 4A). In the developing cortex, the ventricular zone contains the highest levels of LAR expression. Outside of the CNS, LAR is expressed

abundantly in the lung, thymus and skin. The labeling in the lung is punctate and probably associated with the bronchial system. LRP, in contrast, displays a more diffuse labeling pattern throughout the embryo, with approximately equal signals in the CNS and in many non-neural tissues (Fig. 4B). LRP expression within the CNS is also relatively uniform, with slightly higher levels of hybridization along ventricular zones and in the developing cortical plate. Unlike LAR and LRP, RPTPγ labeling is higher in the midbrain, medulla and spinal cord than in the forebrain (Fig. 4C). In the midbrain (Fig. 5C) and cortex, RPTPγ signal is found mostly in regions that contain post-mitotic neurons. At E17, RPTPγ expression is higher in the CNS than in any other tissue in the embryo.

As will be described at length in Chapter 2, CPTP1 is expressed predominantly in the nervous system during the embryonic period. At E17, the highest CPTP1 transcript levels are detected in the developing cortex and midbrain (Fig. 4D). Similarly, P19-PTP mRNA is highest in the cortex and midbrain (Fig. 4E). P19-PTP is also detected diffusely throughout the rest of the embryo.

PTP-1 (Fig. 4F) and SHP (Fig. 4G) are both expressed at highest levels in the developing thymus. A lower, but still detectable level of PTP-1 is found in the cortex, midbrain and spinal cord of the E17 embryo (Fig. 4F). SHP is almost undetectable in the CNS, but is expressed at relatively high levels in the liver (Fig. 4G).

Neurofilament (Fig. 4H), a marker for differentiated neurons, is expressed at high levels in regions of the E17 spinal cord and medulla that contain post-mitotic, post-migratory neurons. It is expressed much less abundantly in the midbrain and cortex. This analysis indicates that expression of the genes for the PTPases begins before postmitotic neurons turn on the

gene for NF. In fact, some of the PTPases are most abundant in regions of high mitotic activity, which do not contain cells that have begun to express NF.

The nervous system expression of the PTPase genes is not restricted to the CNS. While not shown in the sections illustrated here, the dorsal root ganglia (DRGs) express the PTPases examined in the study. In particular, CPTP1, LAR, RPTPγ, P19-PTP and PTP-1 are expressed at high levels in the E17 DRGs (data not shown).

At postnatal day 4, PTPases are preferentially expressed in the gray matter.

Sagittal sections of P4 brains were also examined by in situ hybridization, with the same series of probes used for the embryo sections. At this age, neurogenesis in most areas of the CNS is complete while glia continue to be generated (LeVine and Goldman, 1988). Neurogenesis continues in the cerebellum, where progenitors in the external granular layer give rise to granule cells. Meanwhile, neurite growth and synaptogenesis are ongoing processes throughout the brain.

In the P4 brain, LAR is expressed diffusely with highest levels in the cerebral cortex, hippocampus and cerebellum (Figs. 6A, 7B and 8B). All cortical layers including the subplate express LAR (Fig 7B). The subventricular zone also contains significant LAR mRNA. In the cerebellum, both the external and internal granular layers show LAR hybridization (Figs. 6A and 8B). Hybridization with a sense probe for LAR produces no signal (Fig. 6I).

The distribution of LRP signal is similar to that of LAR (Fig. 6B). All layers of neocortex and the hippocampus display high transcript levels (Fig. 7C). Interestingly, LRP expression in the cerebellum is restricted to the internal granular layer and is absent from the external granular layer (Fig. 8C).

RPTPγ mRNA distribution is markedly different from the other PTPases examined in this study (Figs. 6C, 7D and 8D). First of all, only the superficial layers of cortex contain high levels of RPTPγ mRNA (Figs. 6C and 7D). Furthermore, RPTPγ signal is much higher in the thalamus than in the striatum. Within the thalamus, the ventral and lateral nuclei contain the highest signal. There is no RPTPγ mRNA in the subventricular zone (Figs. 6C and 7D). RPTPγ also shows extensive expression in hippocampus and cerebellum. In the cerebellum, RPTPγ is localized to both external and internal granular layers (Fig. 8D).

The mRNAs for CPTP1 (Fig. 6D), P19-PTP (Fig. 6E) and PTP-1 (Fig. 6F) are distributed in a pattern similar to that of LAR, with the cortex, hippocampus and cerebellum being areas with the highest levels of signal. Within the cortex, all layers including the subplate express all three messages (for CPTP1, see Fig. 7E). In the cerebellum, all of these PTPases are expressed in both the external and internal granular layers (for CPTP1, see Fig. 8E). SHP, on the other hand, is not detectable to any significant degree in P4 brain (Fig. 6G).

DISCUSSION

Using a PCR-based screen, we have characterized seven PTPases that are expressed at P0 in the rat neocortex. Four are receptor PTPases (LAR, LRP, RPTPγ and CPTP1), and three are non-receptor PTPases (PTP-1, P19-PTP and SHP). By northern and in situ hybridization, we have confirmed that the mRNAs encoding these PTPase sequences are indeed expressed in the cortex during embryonic and early postnatal life. This analysis permitted the identification of PTPases that are present at very low levels, such as SHP, as well as relatively abundant PTPases, such as CPTP1.

PTPases are expressed at different relative abundance in the brain.

Although this study was not designed to compare quantitatively the expression of different PTPases, some statements about the relative abundance of these PTPases can be made. Comparisons between the in situ hybridization signals generated with identical amounts of equal length probes for each of the seven PTPases gives an approximation of relative expression levels. For the in situ hybridization, different exposure times were required for optimal detection of each PTPase. These differences reflect the relative abundance of the PTPases. For example, in E17 and P4 brain sections, the CPTP1 signal can be detected after an exposure of 24 hours while P19-PTP and PTP-1 signals can only be detected after 9 days (Figs. 4, 6). LAR, LRP and RPTPγ produce signals of similar intensity after 4.5 days. Therefore, among the seven PTPases analyzed in this study, CPTP1 is likely to be the most abundant PTPase in embryonic and early postnatal brain, followed by LAR, LRP and RPTPγ, expressed at about equal levels. P19-PTP, PTP-1 and SHP are expressed at the lowest levels of any of the PTPases studied here.

Receptor PTPases can transduce extracellular signals in the developing brain.

By northern and in situ hybridization, we have demonstrated that the receptor PTPases, LAR, LRP, RPTPγ and CPTP1, are expressed in the developing central and peripheral nervous systems. Each of these receptor PTPases has a unique extracellular domain and two well-conserved intracellular catalytic domains. All four receptor PTPases described here were previously cloned and sequenced from non-neural tissue. This study is the first report of the spatial and temporal regulation of their expression during neural development. The structure of receptor PTPases suggest that they can function

both as surface recognition molecules and as regulators of protein phosphorylation, which may be critically important during corticogenesis. Identification of their mRNAs and analysis of the spatial and temporal regulation of their expression during neural development can provide insights into their possible functions.

LAR contains three immunoglobulin (Ig)-like repeats and eight fibronectin type III (FN III)-like repeats in its extracellular domain and thus resembles cell adhesion molecules like NCAM (Rutishauser, 1983) and TAG-1 (Furley et al., 1990). While immunocytochemistry with LAR antibodies showed no staining in the brain (Streuli et al., 1992), LAR mRNA can be detected abundantly in this tissue (this study; Longo et al., 1993). In fact, by PCR we find LAR mRNA in the brain as early as E13 (data not shown) and here show a high level of expression in proliferative zones in later embryonic stages. Therefore, LAR could be involved in homophilic interactions that regulate cellular proliferation and/or migration in the nervous system.

Unlike LAR, LRP does not contain cell adhesion molecule-like motifs. Instead, its extracellular domain, composed of 123 amino acids, has multiple glycosylation sites similar to the N-terminal region of CD45. Thus, it has been suggested that LRP may present carbohydrates to lectins (Matthews et al., 1990). There is growing evidence that carbohydrates and lectins are involved in cell recognition and adhesion in the nervous system (Jessell et al., 1990). LRP mRNA is expressed at approximately equal levels throughout cortical development. Moreover, its distribution in the developing cerebellum coincides with the location of differentiated granule cells. Thus, LRP appears to be important for constitutive cellular functions that are not specifically associated with developmental events.

Unlike LRP, RPTPγ has a large extracellular domain. The human RPTPγ

gene maps to a chromosomal region that is frequently deleted in lung and renal cell carcinomas (LaForgia et al., 1991). Its function in the developing brain is far from clear. However, our in situ data indicate that it is expressed by postmitotic neurons in the embryonic midbrain and cortex as well as in the postnatal cerebellum. The up-regulation of RPTPγ during cortical development is consistent with expression by postmitotic cells and may indicate a similar tumor suppressor-like role in the CNS, perhaps to maintain neurons in a non-mitotic state. Interestingly, RPTPγ and a similar PTPase, PTPζ, both have extracellular domains with high homology to the enzyme carbonic anhydrase (Barnea et al., 1993; Krueger and Saito, 1992). The extracellular domain of PTPζ is identical to a proteoglycan which can bind tenascin, NCAM and NgCAM (Barnea et al., 1994a; Barnea et al., 1994b; Grumet et al., 1994). This raises the possibility of these PTPases being involved in cell-cell or cell-matrix interactions during maturation of the CNS.

As we suggested based on partial sequence homology (Sahin and Hockfield, 1993), CPTP1 belongs to the LAR subfamily of receptor PTPases. CPTP1 has been cloned by four groups within the last year and has an extracellular domain that contains three Ig- and five FN III-like repeats suggesting homophilic binding properties (Walton et al., 1993; Pan et al., 1993; Yan et al., 1993). A liver cDNA sequence has three additional FN III-like repeats spliced into the extracellular domain (Zhang et al., 1994). Thus, the liver isoform of CPTP1 has an extracellular region very similar in length and domain structure to LAR. Unlike the six other PTPases described here, CPTP1 is the only one that is more enriched in the adult brain than in non-neural tissues. CPTP1 is expressed as early as E13 in the brain (data not shown), and its relative abundance is higher than any other PTPase that we have examined. Furthermore, it is most abundant in progenitor cells and developing neurons

and may, therefore, be important in the early differentiation of neurons.

Possible roles of PTPases in neural development and function

Because their substrates and extracellular ligands (in the case of the receptor PTPases) have not yet been identified, the cellular functions of PTPases have been difficult to elucidate. However, available evidence indicates that the role of PTPases is more complex than simple reversal of PTK action. The expression patterns of the seven PTPases examined in this study suggest general hypotheses about their cellular functions in the CNS. In the neocortex, CPTP1 and P19-PTP show the highest levels of expression during the period of corticogenesis and then are subsequently down-regulated. LAR is also expressed at a high level in the proliferative zone of the cortex. Cellular interactions during proliferation can regulate the mitotic rate and alter the identity of the progeny (Reh and Kljavin, 1989; Gao et al., 1991; McConnell and Kaznowski, 1991). Thus, the PTPases expressed in the these zones may play roles in the regulation of proliferation and determination of neuronal fate.

In addition, it is becoming increasingly clear that tyrosine phosphorylation is important in regulating neurite outgrowth (Bixby and Jhabvala, 1993; Wu and Goldberg, 1993). Using cultures of embryonic DRGs, which express high levels of PTPases, we have shown that tyrosine phosphorylation affects neurite initiation and outgrowth (Fryer et al., 1993, Soc. Neurosci., abstract). The receptor PTPases, with their putative roles as cell adhesion and transmembrane signaling proteins, would be well suited to mediate surface recognition and axonogenesis. In contrast, other PTPases (LRP, RPTPγ and the high molecular weight transcript for LAR) are expressed either at a constant level or at increasing levels through postnatal life, implying that they play a constitutive role in mature neural functions. Consistent with this

observation, recent studies in Aplysia indicate that PTPases can regulate the function of ion channels and thus affect the excitability of mature neurons (Wilson and Kaczmarek, 1993). Results from other groups implicate tyrosine phosphorylation in the control of mammalian ion channels as well (reviewed in Siegelbaum et al., 1994).

Because of the complexity of cellular interactions in the developing CNS, a complete understanding of the role of PTPases in neural development still remains in the future. The diversity and abundance of PTPase genes expressed in the brain provides compelling evidence that PTPases are important players in the complex cell-cell interactions that regulate cell fate determination in the CNS. We believe that we still have not explored the full repertoire of PTPases expressed in the developing cortex. While a low-stringency PCR screen is quite sensitive for identifying rare messages, it is neither quantitative nor exhaustive because of the bias introduced by the choice of primers. This is demonstrated by the fact that at least two PTPases that are known to be expressed in developing cortex were not detected here: PTPζ (also known as RPTPβ; Krueger and Saito, 1992; Levy et al., 1993) and syp (Feng et al., 1993). The results we present here, however, indicate that PTPases are important in neurogenesis. This detailed characterization of the expression of several PTPases in the CNS will now permit further genetic and biochemical analyses that will help decipher their precise roles in neural development.

Figure 1. PCR primers were constructed from the most conserved regions in the catalytic domain.

A. Three members of the transmembrane PTPase family are shown to illustrate the extent of similarity in their first phosphatase domains: human CD45 (Streuli et al., 1987), human LAR (Streuli et al., 1988) and mouse LRP (Matthews et al., 1990). The alignments were produced using the BESTFIT function of the GCG program (Genetics Computer Group, 1991). Amino acids conserved among the three PTPases are indicated in bold. Regions selected for primers are shown in boxes.

B. Amino acid sequence used to generate the PCR primers.

C. Degenerate nucleic acid sequence of the primers.

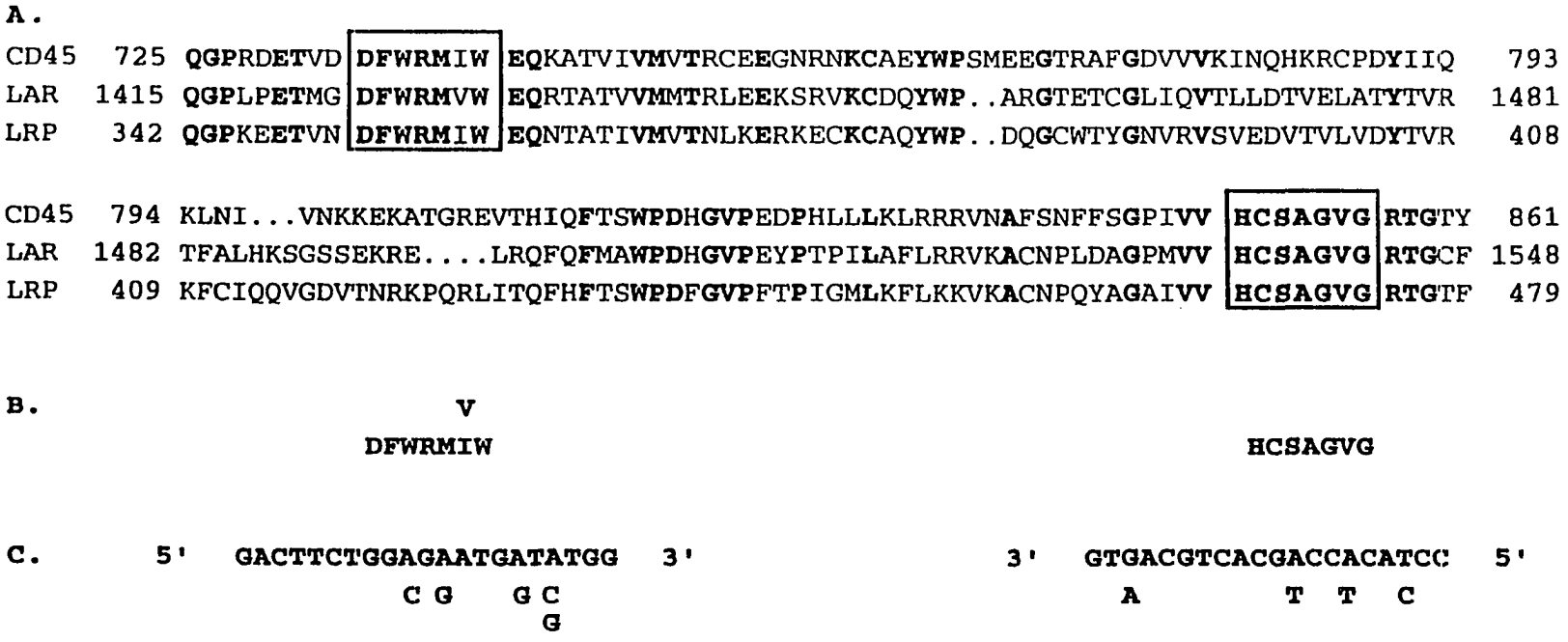

Figure 2. PCR from a P0 cortex library resulted in the identification of seven PTPase domains. Deduced amino acid sequences of the PCR products are shown. Sequences were aligned using the PILEUP function of the GCG program (Genetics Computer Group, 1991). Amino acids encoded by the PCR primers are not shown. Residues conserved among all seven clones are shown in black boxes. The intensity of gray shading indicates the degree of amino acid similarity. The order of the sequences reflects the level of homology between sequences, with highest homology at the top. The first four sequences belong to receptor PTPases while the last three belong to non-receptor PTPases. The clones are identical or highly similar to the sequences reported in the following references: LAR (Pot et al., 1991; Yu et al., 1992); LRP (Moriyama et al., 1992); RPTPγ (Barnea et al., 1993); CPTP1 (Sahin and Hockfield, 1993; Walton et al., 1993; Pan et al., 1993; Yan et al., 1993); P19-PTP (den Hertog et al., 1992; Yang et al.,1993; Sahin and Hockfield, 1993); PTP-1 (Guan et al., 1990); SHP (Matthews et al., 1992; Yi et al., 1992). The second catalytic domains of LAR and CPTP1 that were also identified in this screen are not shown here.

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Cptp1    EQRSATVVMM  TRLEEKSRVK  CDQYWPNRGT  ETY....GFI  QVTLL
Lar      EQRTATVVMM  TRLEEKSRVK  CDQYWPARGT  ETY....GLI  QVTLV
Rptpg    EQNTGIIIMI  TNLVEKGRRK  CDQYWPTENT  EEY....GNI  IVTLK
Lrp      EQNTATIVMV  TNLKERKECK  CAQYWPDQGC  WTY....GNV  RVSVE
Ptp-1    EQKSRGVVML  NRIMEKGSLK  CAQYWPQKEE  KEMVFDDTNL  KLTLI
Shp      QENTRVIVMT  TREVEKGRNK  CVPYWPEVGT  QRVY...GLY  SVTNC
P19-Ptp  EYNVVIIVMA  CREFEMGRKK  CERYWPLYGE  DPITF..APF  KSSCE

Cptp1    DTMELATSCV  RTFSLHK...  .........N  SSEKREVRHF  QTAW
Lar      DTVELATYTM  RTFALHK...  .........S  SSEKRELRQF  QMAW
Rptpg    STEVLACYTV  RRFSVRNTKV  KKGQKGNPKG  RQNERTVIQV  HYTQW
Lrp      DVTVLVDYTV  RKFCTQQV..  .....GDVTN  RKPQRLITQF  HTSW
Ptp-1    SEDVKSYYTV  RQELENL...  .........A  TQEAREILHF  HYTTW
Shp      KEHDTAEYKL  RTQTSPL...  .........D  NGDLVREIWH  QYLSW
P19-Ptp  NEQARTDYFS  RTLLE....  .........F  QNESRRLYQF  HYVNW

Cptp1    PDHGVPEYPT  PFLAFLRRVK  TCN..PPDAG  PMVV
Lar      PDHGVPEYPT  PILAFLRRVK  ACN..PLDAG  PMVV
Rptpg    PDMGVPEYAL  PVLTFVRRSS  AAR..MPDMG  PLV
Lrp      PDFGVPFTPI  GMLKFLKKVK  ACN..PQYAG  AIVV
Ptp-1    PDFGVPESPA  SFLNFLFKVR  ESGSLSPEHG  PIVV
Shp      PDHGVPSEPG  GVLSFLDQVN  QRQESLPHAG  PIVV
P19-Ptp  PDHDVPSSFD  SILDMISLMR  KYQE..HEDV  PIC
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Figure 3. Northern hybridization demonstrates that different PTPases have different developmental and spatial expression patterns. A. 25 μg of total RNA extracted from cerebral cortex from animals at embryonic day 16 (E16), postnatal days (P) 0, 4, 14, 30 and adult (AD) as well as from P35 liver (LIV) were run in formaldehyde-agarose gels and blotted onto nitrocellulose. The same blot was used for seven rounds of hybridization with six PTPase probes and a probe for cyclophilin. The LAR blot shown is of the same RNA samples, but run on a separate blot. Equal loading of lanes was confirmed by probing both blots with cyclophilin (CYCLO), which showed the same loading levels on both blots. Multiple transcripts of several receptor PTPases are detected in the cerebral cortex (LAR, RPTPγ, CPTP1). Some PTPases are down-regulated (CPTP1, P19-PTP) over the course of cortical development while others are up-regulated (LAR, RPTPγ). **B.** 20 μg of total RNA isolated from various adult rat tissues and five cell lines was loaded in each lane. Tissues are: heart (HE), kidney (KID), lung (LU), whole brain (BR), thymus (THY), spleen (SPL), muscle (MUS), pancreas (PAN), liver (LIV), testis (TES), ovary (OV). Cell lines are: L929 mouse fibroblasts (FIB), WEHI3 mouse myeloid leukemia cells (MyL), NF8D5 mouse lymphocytic leukemia cells (LyL), GC rat pituitary cells (GC) and YAC mouse lymphocytic leukemia cells (YAC). The positions of 28S and 18S rRNAs are indicated by large and small arrowheads, respectively. Two separate blots that contained almost all the same RNA samples were used in this analysis. Equal loading of the lanes was confirmed by ethidium bromide staining and photographing the gel (the bottom panel). This analysis demonstrates that some PTPases are present at high abundance in the adult brain: LRP, RPTPγ and CPTP1. Others are barely detectable in brain (SHP). Of these PTPases, only CPTP1 appears to be substantially enriched in the nervous system relative to non-neural tissues.

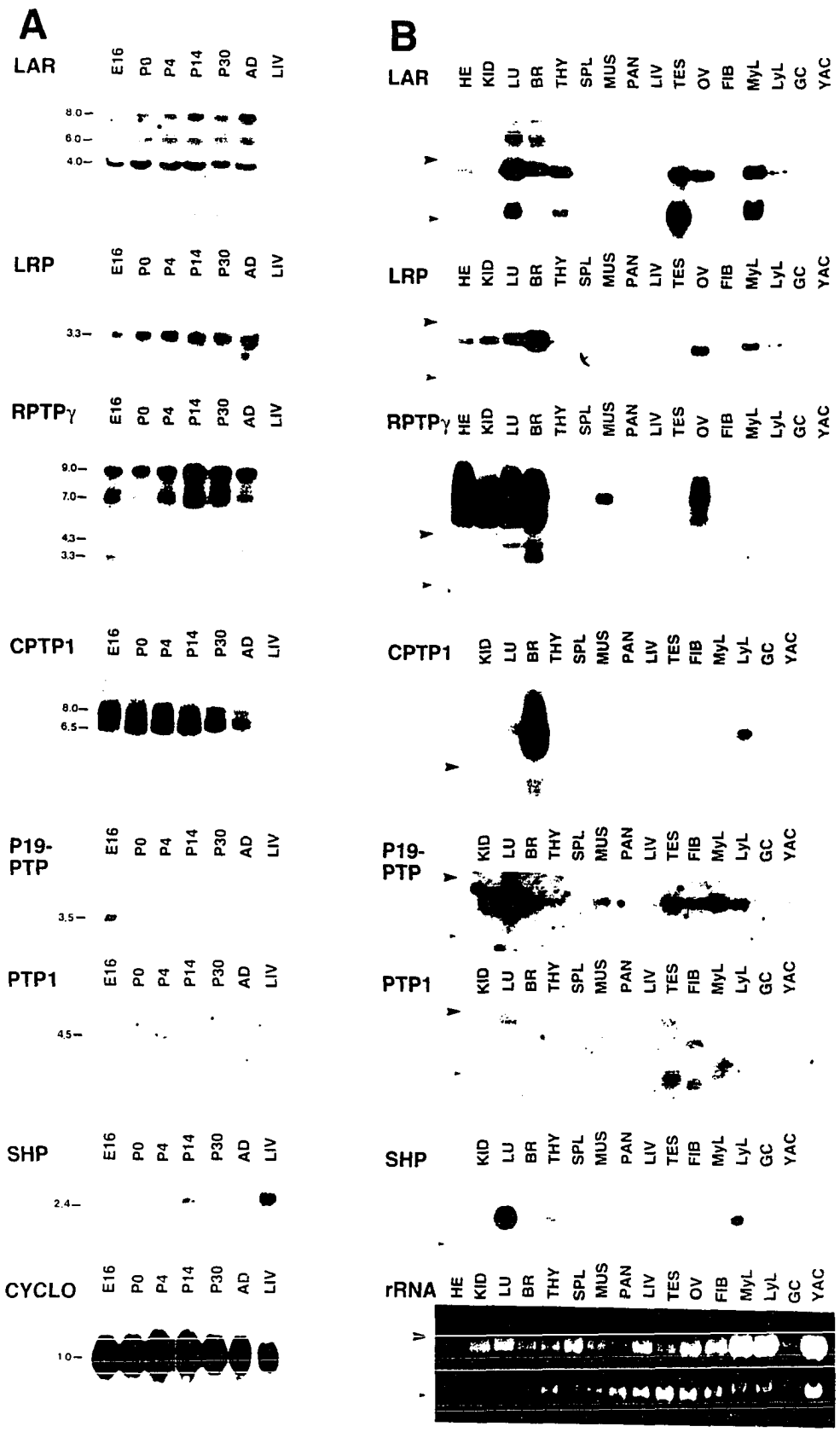


Figure 4. In situ hybridization demonstrates that PTPases are expressed in the CNS during embryonic development.

Near adjacent parasagittal sections of E17 animals were hybridized to ^{35}S-labeled antisense RNA probes for (A) LAR, (B) LRP, (C) RPTPγ, (D) CPTP1, (E) P19-PTP, (F) PTP-1, (G) SHP, (H) NF as a positive control and (I) sense probe for LAR as a negative control, and exposed to film. **(A)** At E17, a high LAR signal is detected in the cerebral cortex (c), midbrain (mb), spinal cord (s), lung (lu) and thymus (t). In the cortex, the labeling is highest in the proliferative zone immediately surrounding the lateral ventricle (v) and is much lower near the pial surface where postmitotic neurons reside. **(B)** LRP mRNA is present in both neural and non-neural structures. In the CNS, the highest level of labelling is seen in the cortex. **(C)** RPTPγ mRNA is expressed at the highest levels in the midbrain and medulla. There is less, but still detectable labeling in the cortex and the spinal cord. **(D)** CPTP1 signal is detected at high levels in the cortex, midbrain and spinal cord. Both the ventricular zone and the cortical plate show high levels of CPTP1 signal. **(E)** P19-PTP mRNA is found at highest levels within the cortex surrounding the lateral ventricle and in the midbrain. **(F)** PTP-1 signal is present at a low level throughout the CNS and at somewhat higher levels in the thymus. **(G)** SHP is virtually undetectable in the CNS, while high levels are present in the liver (liv) and thymus. **(H)** At E17, NF mRNA is expressed at higher levels in the spinal cord than the more rostral parts of the CNS. The midbrain and the cortex display very little signal. **(I)** The sense LAR probe, the negative control, shows no signal. Exposure times for these autoradiograms are as follows: CPTP1 (D) - 1 day; LAR (A) , LRP (B), RPTPγ (C), NF (H) - 4.5 days; P19-PTP (E), PTP-1 (F), SHP (G), sense control (I) - 9 days. (Scale bar = 1 mm)

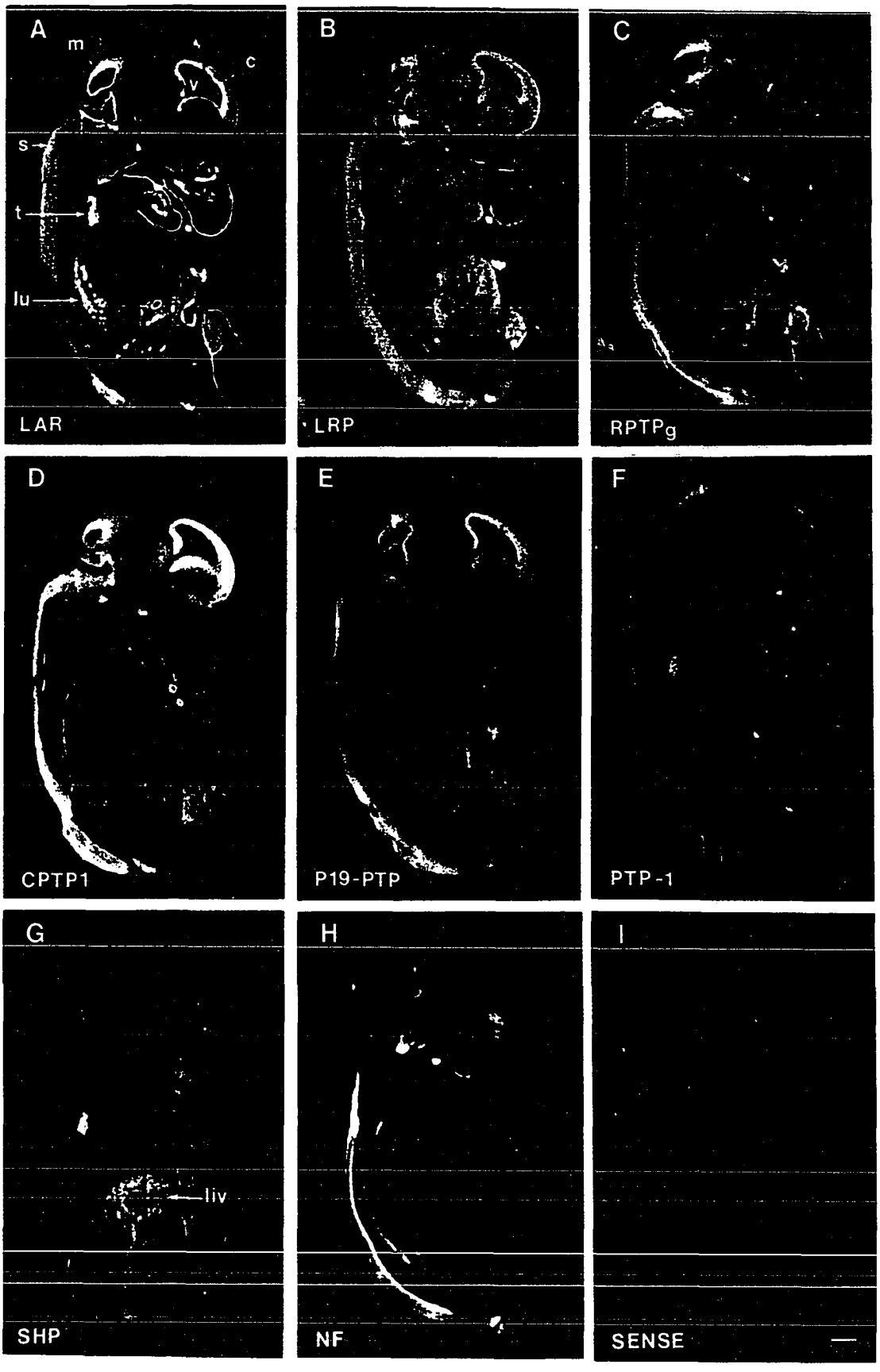


Figure 5. In contrast to the other PTPases, RPTPγ is expressed predominantly by post-mitotic neurons. Emulsion dipped slides were counter stained with cresyl violet and photographed under bright-field optics for cell localization (A) or under dark-field optics for visualization of silver grains generated by probes to LAR (B), RPTPγ (C), and NF (D). **(A)** Parasagital section through the midbrain on E16 shows the different layers of the developing midbrain. The pial surface is toward the top; the ventricular surface is toward the bottom. The ventricular zone (v) is densely populated by proliferating cells while the zone of post-mitotic cells (arrow) is relatively cell sparse. **(B)** A corresponding section labeled with antisense LAR probe demonstrates that LAR mRNA is detected in both the mitotic and post-mitotic zones of the midbrain. **(C)** In contrast, RPTPγ mRNA is predominantly localized to the region of post-mitotic neurons (arrow) and is almost absent from the ventricular zone. **(D)** Similarly, NF mRNA is abundant in the post-mitotic zone (arrow), but is absent from the venricular zone, consistent with its expression by post-mitotic, differentiated neurons. (Scale bar = 50 μm)

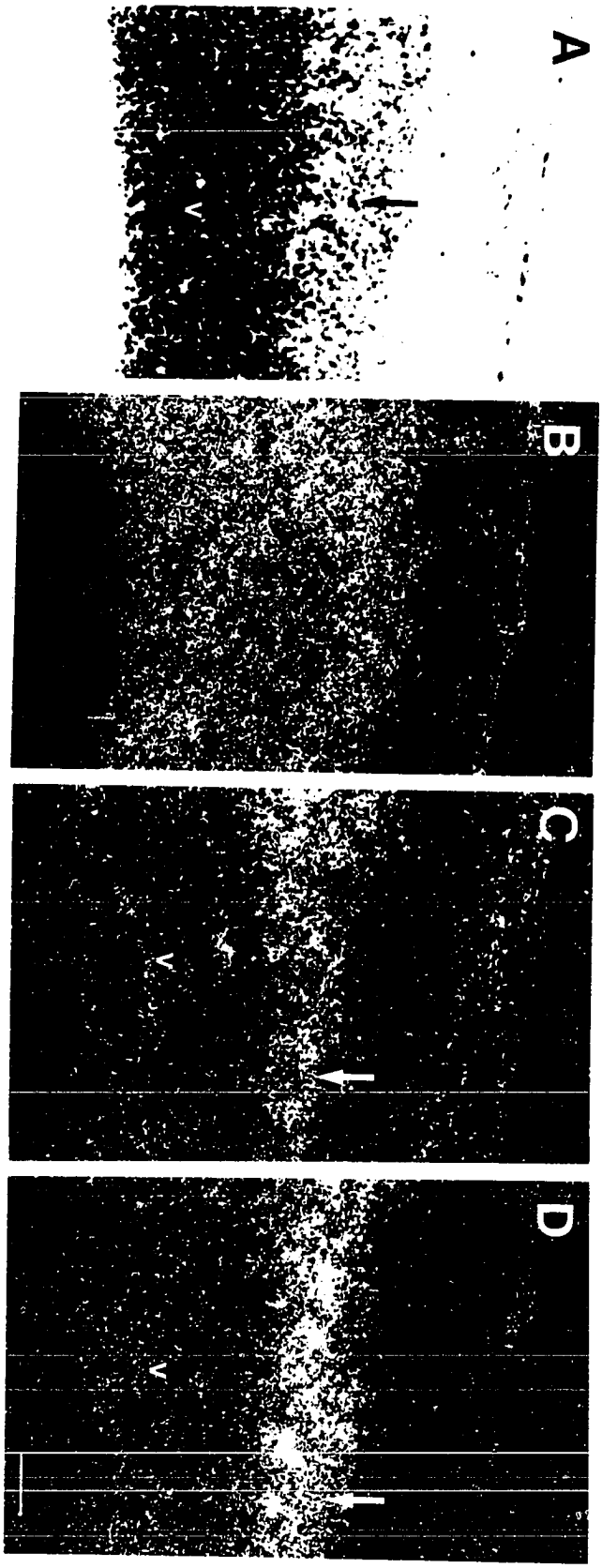


Figure 6. PTPases are expressed in the early postnatal brain. Parasagittal sections from P4 brains were hybridized to ^{35}S-labeled antisense RNA probes for (A) LAR, (B) LRP, (C) RPTPγ, (D) CPTP1, (E) P19-PTP, (F) PTP-1, (G) SHP, (H) NF as positive control and (I) sense probe for LAR as negative control, and exposed to film. **(A)** At P4, a high level of LAR signal is detected in the neocortex (c), cerebellum (cb) and hippocampus (h). In the neocortex, all layers including the subplate (arrow) contain LAR signal. (See also figure 6.) **(B)** LRP signal is similar to that of LAR; it is most intense in the neocortex, hippocampus and cerebellum. **(C)** RPTPγ mRNA is detected in a pattern different from LAR and LRP. Signal is most intense in the neocortex, hippocampus, thalamus (t) and cerebellum. Within the neocortex, the superficial layers contain the highest levels of RPTPγ signal. (See also figure 6.) **(D)** CPTP1 is abundant in neocortex, hippocampus, and cerebellum. The highest levels of P19-PTP **(E)** and PTP-1 **(F)** mRNA are also detected in the same regions as CPTP1. **(G)** SHP message is detected at very low levels in the brain. **(H)** NF is abundant in hippocampus, thalamus, cerebellum and brainstem. Within the neocortex, the highest signal is in the middle layers. **(I)** Sense probe for LAR shows no hybridization signal. Exposure times for the sections are as follows: CPTP1 (D), NF (H) - 1 day; LAR (A) , LRP (B), RPTPγ (C) - 4.5 days; P19-PTP (E), PTP-1 (F), SHP (G), sense control (I) - 9 days. (Scale bar = 1 mm)

Figure 7. Receptor PTPases in postnatal cortex. Emulsion dipped slides were counter stained with cresyl violet and photographed under bright-field optics for cell localization (A) or under dark-field optics for visualization of silver grains generated by probes for LAR (B), LRP (C), RPTPγ (D) and CPTP1 (E). **(A)** Parasagital section of P4 cortex counter stained with crestyl violet shows the different layers of neocortex (II-VI) and the subplate (s), hippocampus (h), subventricular zone (svz; Luskin, 1993) and the white matter (wm). **(B)** A near adjacent section of P4 cortex hybridized with radiolabeled antisense LAR probe demonstrates that LAR mRNA is found in all layers of neocortex and hippocampus (h), as well as in the subventricular zone (svz). The subplate (arrow), while a relatively cell sparse layer, shows intense hybridization signal. There is no labeling in the white matter (wm). **(C)** LRP labeling is similar with highest levels detected in the hippocampus (h) and neocortex. **(D)** RPTPγ is also expressed in the hippocampus (h). However, in contrast to other PTPases, RPTPγ mRNA is far more abundant in the superficial layers of neocortex than in the deeper layers. The subplate also contains detectable labeling (arrow), however, the subventricular zone (svz) is virtually unlabeled by RPTPγ. **(E)** CPTP1 mRNA is expressed abundantly in the neocortex, hippocampus (h) and subventricular zone (svz). (Scale bar= 200 μm)

Figure 8. Receptor PTPases in postnatal cerebellum. (A) An emulsion dipped slide counter stained with cresyl violet and photographed under bright-field optics demonstrates the cell layers in the P4 cerebellum: external granular layer (EGL), Purkinje cell layer (PC), internal granular layer (IGL). The EGL contains proliferating granular cell precursors while the IGL contains the post-mitotic, post-migratory granular cells. **(B)** LAR mRNA is found in both the EGL and the IGL. **(C)** In contrast to other receptor PTPases, LRP is expressed exclusively in the IGL (double arrows). **(D)** RPTPγ mRNA is detected in both the EGL (single arrow) and the IGL (double arrows). **(E)** Similarly, CPTP1 is found in the deep EGL (single arrow) and also in the IGL (double arrows). (Scale bar= 100 μm)

CHAPTER 2

TWO PROTEIN TYROSINE PHOSPHATASE GENES, CPTP1 AND P19-PTP, ARE EXPRESSED AT THEIR HIGHEST LEVELS DURING NEUROGENESIS AND EARLY NEURONAL DIFFERENTIATION

SUMMARY

The first chapter described a PCR-based approached that allowed us to identify PTPase genes expressed in the rat cerebral cortex at birth. At the time of our study (February, 1992), three of the clones encoding putative PTPase domains were not identical to any sequences in the GenBank. We referred to these clones as Cortex-enriched Protein Tyrosine Phosphatases (CPTPs) 1, 2 and 3 (Sahin and Hockfield, 1993). In this chapter, we examine the expression patterns of these three clones by northern and in situ hybridization. This analysis demonstrates that all three clones are enriched in the nervous system during embryonic and early postnatal days. Then, we provide evidence that CPTP1 and CPTP3 represent the two phosphatase domains of a single receptor PTPase gene, which we call CPTP1. Since the completion of our study, a full length murine cDNA sequence named P19-PTP, which is highly homologous to CPTP2, has been reported (Den Hertog et al., 1992). Thus, in this chapter we refer to this non-receptor PTPase gene as P19-PTP.

INTRODUCTION

Tyrosine dephosphorylation has been associated with cellular differentiation in a number of non-neural tissues. For example, during granulocytic differentiation of leukemia cell lines, phosphotyrosine residues decrease while PTPase activity increases (Frank and Sartorelli, 1988). There is growing evidence that dephosphorylation may play a significant role in differentiation of neural tissues as well. For example, NGF-induced neuronal differentiation of PC12 cells is associated with an increase in PTPase activity (Aparicio et al., 1992). In the Drosophila nervous system, three receptor PTPases are selectively expressed on subsets of developing axons (Yang et al., 1991; Tian et al., 1991; Hariharan et al., 1991). Perhaps of greatest interest in regard to possible roles in cell-cell signaling, many receptor PTPases (including those identified in the Drosophila nervous system) have fibronectin type III (FN-III) and/or immunoglobulin (Ig)-like domains in their extracellular domains, suggesting that they may have dual functions: cell adhesion and signal transduction.

While few PTPases have been reported in the developing brain, the level of tyrosine phosphorylation indicates that PTPases must be particularly active early in neurogenesis (Maher, 1993). Furthermore, the increasingly large number of PTKs with demonstrated expression during neural development (Lai and Lemke, 1991; Sanchez et al., 1994) implies that there may also be a large group of PTPases involved in neuronal growth and differentiation. To identify PTPases expressed in neonatal rat cortex, we performed PCR amplification under low stringency annealing conditions with primers corresponding to the conserved catalytic domains of previously reported receptor PTPases (Sahin and Hockfield, 1993; also see Chapter 1). In this analysis, three clones

encoding putative PTPase domains contained sequences that were not identical to any PTPase genes in the databases. They also lacked significant identity to one another. We referred to these clones as Cortex-enriched Protein Tyrosine Phosphatases (CPTPs) 1, 2 and 3.

At the time of our study, the deduced amino acid sequences of CPTP1 and CPTP3 showed the highest homology to human receptor PTPases LAR (Streuli et al., 1988) and HPTPδ (Krueger et al., 1990). CPTP1 was highly homologous to the first phosphatase domain of human LAR and HPTPδ, while CPTP3 was almost identical to their second catalytic domains (Sahin and Hockfield, 1993). These data indicate that CPTP1 and CPTP3 belong to the LAR subfamily of receptor PTPases, but neither is identical to rat LAR.

On the other hand, the sequence of CPTP2 had the closest similarity to a non-receptor PTPase, PEP (PEST-domain phosphatase) (Matthews et al., 1992). During the preparation of our study, a full cDNA sequence of a phosphatase from murine P19EC cells (named P19-PTP) appeared in the GenBank. At the nucleic acid level, CPTP2 shows 93% homology to P19-PTP. The deduced peptide sequence of the phosphatase domain of P19-PTP is identical to the CPTP2 peptide sequence except for one amino acid (Den Hertog et al., 1992). The tissue distribution and developmental regulation of P19-PTP has not been reported, but based on size and sequence information, P19-PTP is likely to be the murine homologue of CPTP2.

In this chapter, we use the three CPTP clones as probes for northern and in situ hybridization to examine the expression patterns of their respective genes. This analysis demonstrates that the expression of all three PTPs is enriched in the nervous system during the embryonic and early postnatal periods. Then, we provide evidence that CPTP1 and CPTP3 represent the two phosphatase domains of a single receptor PTPase gene, which we call CPTP1.

The temporal and spatial regulation of expression of the mRNAs identified by these sequences suggests that they encode proteins that may participate in neuronal development.

MATERIALS AND METHODS

PCR Amplification

PCR amplification from cDNA was essentially performed as described Chapter 1. The degenerate oligonucleotide primers used here were the same as those used in the previous study and correspond to the conserved catalytic domain amino acid sequences DFWRM(I/V)W (upstream) and HCSAGVG (downstream). A postnatal day zero (P0) rat occipital neocortex cDNA library was used as the DNA template for the amplification (Sahin and Hockfield, 1993). Twenty five pmol each of sense and antisense primers were used in 25 µl reactions in *Taq* buffer (10 mM Tris-HCl pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% gelatin) with 0.6 units of *Taq* polymerase, 200 µM dNTPs and 25 ng of cDNA library as template. PCR was carried out in a Perkin Elmer DNA Thermal Cycler for 35 cycles. Each cycle included a 30 sec denaturation at 94°C and a 3 min extension at 72°C. In order to facilitate the annealing of the degenerate primers, the initial five cycles included a 1 min annealing step at 37°C and a slow ramp (1°C per 4 sec) between annealing and extension. The subsequent 30 cycles utilized 1 min annealing step at 45°C with no ramp. One microliter aliquots of the reaction were used to ligate the amplified fragments into the TA vector (Invitrogen). Plasmids with inserts were chosen by blue/white selection and examined by PCR with the original degenerate primers for the presence of PTPase domains. Inserts that gave a band of the appropriate size (350 bp) after PCR were sequenced by the dideoxy chain termination method (Sequenase, U.S. Biochemical) on both strands using M13 (-40) forward and reverse

primers. Sequence analyses were conducted using GCG software (Genetics Computer Group, 1991). The PCR clones were then used for the northern and in situ hybridization analyses as described below.

RNA isolation and Northern hybridization

Total cellular RNA was extracted from various tissues of Sprague-Dawley rats using the guanidine thiocyanate/cesium chloride ultracentrifugation method (Bothwell et al., 1990). For the analysis of developmental regulation, the cerebral cortex was dissected from animals at embryonic day 16 (E16), postnatal day 0 (P0), P4, P14, P30 and adult, using care to exclude the underlying diencephalon and the hippocampus. RNA was similarly extracted from P0 and P35 liver; P35 kidney; P14 and adult spinal cord. Total (25μg) or poly A^+ (1-2 μg) RNA was denatured in 2.2 M formaldehyde, 50% formamide, 1X MOPS buffer at 65°C for 15 min. RNA was resolved by electrophoresis on a 1.0% agarose gel containing 2.2 M formaldehyde and 1X MOPS buffer, transferred to Zeta-probe (Bio-Rad) by capillary blotting and then baked (80°C) under vacuum for 2 hours. Hybridization was carried out in 7% SDS, 1% BSA, 0.5 M phosphate buffer pH 6.8 (PB), 1mM EDTA for at least 8 hours at 65°C (Church and Gilbert, 1984). Hybridization solution contained 1-3 x 10^6 cpm/ml of probe made by random primed labeling of the PCR fragments for each PTPase clone (Boehringer Mannheim). For random priming, PCR fragments were either gel purified using GeneClean (American Bioanalytical) or isolated using Magic PCR Preps (Promega). After labeling with [^{32}P]-dCTP (Amersham), the specific activity of the probes were 2-8x10^8 cpm/μg. After hybridization, filters were washed twice in 5% SDS, 0.5% BSA, 40 mM PB, 1 mM EDTA and four times in 1% SDS, 40 mM PB, 1 mM EDTA at 65°C for 20 min. Cyclophilin, which is present at a constant abundance throughout development (Lenoir et

al., 1986), was used as a control for equal loading of lanes. RNA molecular weight standards (GIBCO/BRL) were included on the blots to estimate the sizes of the transcripts. Densitometry of the autoradiograms was performed on the LKB Ultrascan XL system.

In situ hybridization

In situ hybridization was performed as in Martin et al. (1992) with minor modifications. Twelve micron frozen sections were thaw-mounted onto gelatin coated slides, post-fixed and dehydrated. Sections were prehybridized in 2X SSC, 50% formamide at 50°C for 1 hour. Tissues were then hybridized in 50% formamide, 1X Denhardt's, 0.75 M NaCl, 10% dextran sulfate, 15 mM DTT, 10 mM Tris-HCl pH 7.5, 1 mM EDTA, 0.5 mg/ml tRNA, 100µg/ml salmon sperm DNA and 1-2 x10^6 cpm probe at 50°C for 8-12 hours. The [^{35}S]-CTP (NEN) labeled probes were generated using the T7 and SP6 promotors in the TA vector and the Riboprobe system (Promega). Neurofilament-middle (NF-M) antisense and CPTP3 sense probes were used as positive and negative controls, respectively (Martin et al., 1992). The negative control did not give a signal. Following hybridization, the slides were treated with 20 µg/ml RNase A at 37°C for 30 min. Final washes were done in 0.1X SSC, 0.1% β-mercaptoethanol at 65°C for 30 min. Slides were then exposed to Kodak XAR film for 24-120 hrs. Autoradiograms were used as negatives to print figures. To analyze the signal at higher resolution, the slides were dipped into emulsion, developed after 30-90 days and counter stained with cresyl violet.

RESULTS

CPTPs recognize developmentally regulated mRNAs in the rat neocortex.

The size, tissue distribution and developmental expression of RNA species encoding the CPTPs were determined by northern blot analysis using the identified phosphatase domains. RNA samples isolated from rat neocortex at different embryonic and postnatal ages, as well as postnatal spinal cord, liver and kidney, were probed with radiolabeled PCR fragments for each CPTP. Confirmation of equal loading of the lanes was obtained by reprobing the blots for cyclophilin. Densitometry was used to determine the temporal regulation of the CPTP transcripts in the brain, where the ratio of absorbance of individual CPTP bands to that of the cyclophilin band was calculated for each RNA sample. Cyclophilin was used as a control because it is present at a constant relative abundance throughout brain development (Lenoir et al., 1986), in contrast to actin or tubulin whose relative abundance in the brain varies with age (Schmitt et al., 1977; Geschwind and Hockfield, 1989). Cyclophilin is not, however, expressed at equivalent levels in different organs. For instance, it is expressed at lower levels in the liver than in the brain (Danielson et al., 1988).

For CPTP1, two prominent transcripts are detected in the rat cortex, 6.5 kb and 7.8 kb (Fig. 1A). The 7.8 kb message is preferentially expressed in the CNS, but is also weakly expressed in the liver and kidney. In the cortex, this message is the predominant transcript during embryonic life and is found at high levels at embryonic day (E16). The expression of the 7.8 kb transcript falls by postnatal day four (P4) and is relatively constant thereafter (Fig. 1E). In contrast, the 6.5 kb message is not detected in the E16 cortex. Its expression begins around birth, quickly approaches that of the 7.8 kb species during early postnatal days and remains relatively constant throughout the remainder of postnatal life (Fig. 1E). The 6.5 kb band appears to be neural tissue specific; it is not detected in RNA samples from adult liver (Fig. 1A). This species is similarly absent in RNA from adult kidney and P0 liver (data not shown). Both transcripts

for CPTP1 are detected in postnatal spinal cord at levels similar to those found in postnatal cortex (data not shown).

For P19-PTP (CPTP2), a single mRNA species of 3.5 kb is observed (Fig. 1B). In the cortex, this transcript is detectable at high levels at E16. The level of cortical expression declines around birth and remains close to adult levels after P4 (Fig. 1E). The level of expression of P19-PTP in adult cortex is similar to that in the adult liver (Fig. 1B), spinal cord and kidney (data not shown).

RNA detected with CPTP3 parallels CPTP1 in size, tissue distribution, and developmental regulation (Fig. 1C and 1E). The CPTP3 probe hybridizes to two prominent bands at 6.5 and 7.8 kb. While the 7.8 kb band is more abundant in prenatal cortex, both bands are expressed at equal levels postnatally. The 6.5 kb form is not found in the liver. As discussed further below, the correspondence between the transcripts detected by CPTP1 and CPTP3 suggest that they may represent the two phosphatase domains of a single PTPase gene.

CPTPs are expressed in the CNS during embryonic and postnatal development.

To determine the spatial distribution of CPTP mRNAs during development, we performed in situ hybridization using radiolabeled antisense RNA. Both embryonic and postnatal CNS tissues were analyzed by this method. Near adjacent sections were probed with neurofilament-middle (NF-M) as a positive control for neuronal RNA expression.

CPTP1: During embryogenesis, CPTP1 expression is higher in the nervous system than in non-neural tissues. CPTP1 mRNA is detected in E15 embryos at high levels in the CNS (Fig. 2A). At this stage, CPTP1 mRNA is widely distributed in the CNS including the cortex, midbrain, medulla and spinal

cord and peripherally in the dorsal root ganglia (DRG). At E15, many parts of the CNS do not exhibit a strong hybridization signal to NF-M probe (Fig. 2D). Thus, CPTP1 appears to be expressed before neurons start expressing neurofilament. By E17, CNS expression of CPTP1 has increased, with highest transcript levels detected in the developing dorsal telencephalon and midbrain (Fig. 2E). Around the lateral ventricles, the level of CPTP1 expression is higher in the developing cortex than in the ganglionic eminence. More caudal regions of the nervous system such as the spinal cord and DRGs express CPTP1, but at somewhat lower levels than in the cortex and midbrain.

During postnatal development, CPTP1 transcripts are detected non-uniformly in the brain. At P4, the highest levels of CPTP1 are found in the neocortex, hippocampus and cerebellum (Fig. 3A). Within the neocortex, CPTP1 mRNA appears most abundant in the superficial layers and the subplate (Figs. 3A and 4B). This hybridization pattern is markedly different from the pattern of NF-M expression, where hybridization is most intense in the middle layers of the neocortex (Fig. 3D), correlating with a higher neuronal density. CPTP1 and NF-M also show different patterns of expression in the developing cerebellum. At P4, CPTP1 is expressed in both the external and internal granular layers (Figs. 3A and 4D) while NF-M appears to be excluded from the external granular layer (Fig. 3D). CPTP1 expression in the adult neocortex is lower than at P4 while adult hippocampus and entorhinal cortex continue to express this message at significant levels (Fig. 3E).

The expression of CPTP1 in the spinal cord follows a similar developmental regulation to that in the forebrain. CPTP1 is expressed throughout the rostro-caudal extent of the spinal cord at embryonic ages (Fig. 2E). At P4, CPTP1 is expressed in the spinal gray matter, with somewhat higher levels of expression in the dorsal horn (Fig. 5A). NF-M is expressed at higher

levels in the ventral than in the dorsal horn (Fig. 5D). At higher magnification, CPTP1 signal is seen associated with neurons (Fig. 4F). In the adult spinal cord, CPTP1 expression is diminished relative to early postnatal levels, but remains higher in gray matter than in white matter (Fig. 5E).

P19-PTP (CPTP2): P19-PTP mRNA is widely distributed in the embryo, but the highest in situ signals are found in the CNS, DRGs and liver. Within the CNS at E15 and E17, the telencephalon exhibits the strongest hybridization (Figs. 2B and 2F). Postnatally, neocortical P19-PTP expression is markedly down-regulated. At P4, the P19-PTP message is still expressed in the cerebellar cortex, but there is only a weak and diffuse signal present in the neocortical gray matter (Fig. 3B). This low level of expression persists in the adult brain (Fig. 3F). Within the spinal cord, P19-PTP is found at high levels in the gray matter during early postnatal life. Hybridization is seen in both ventral and dorsal horns at P4 (Fig. 5B). In the adult spinal cord, the signal is less intense although still restricted to the gray matter (Fig. 5F).

CPTP3: The hybridization pattern of CPTP3 in the CNS generally follows the pattern of CPTP1. In the embryonic CNS, CPTP3 mRNA is detected at highest levels in the cortex and midbrain at both E15 and E17 (Figs. 2C and 2G). Neocortical expression of CPTP3 persists into early postnatal life. At P4, the hippocampus and cerebellum also show strong hybridization to CPTP3. The CPTP3 expression in the cerebellum appears to be highest in the external and internal granular layers, similar to that seen with CPTP1 (Fig. 3C). In the adult, a diffuse signal is seen in the neocortex and hippocampus (Fig. 3G). In the spinal cord, CPTP3 is found throughout the gray matter at P4 (Fig. 5C). The distribution of the signal remains the same, but the intensity declines in the adult spinal cord (Fig. 5G).

CPTP1 and CPTP3 represent the two phosphatase domains of a single PTPase gene.

Virtually all transmembrane PTPases have two conserved intracellular phosphatase domains. The similarity between the first phosphatase domains of different PTPases is higher than that between first and second domains within any single phosphatase. The same is true for the second phosphatase domains. Interestingly, CPTP1 is highly homologous to the first, and CPTP3 to the second, catalytic domains of LAR and HPTPδ. Moreover, CPTP1 and CPTP3 have almost identical developmental expression profiles on northern blots and very similar patterns of expression by in situ hybridization. These observations suggest that CPTP1 and CPTP3 may represent the first and second catalytic domains of a new receptor PTPase. In order to verify this possibility, PCR was used to screen for cDNAs that contain both CPTP1 and CPTP3. A non-degenerate oligonucleotide primer internal to CPTP1 (corresponding to amino acids LATFCVR) and the degenerate downstream PTPase primer (see Chapter 1) were used to amplify cDNAs from the P0 library. This amplification resulted in two PCR products with approximate molecular weights of 100 and 1100 bps (data not shown). Subcloning and sequencing of the large PCR product revealed that it contained CPTP1 sequences on the 5' end and CPTP3 sequences on the 3' end. The region in between CPTP1 and CPTP3 also showed high homology to rat and human LAR and to HPTPδ (82, 84, 88% amino acid identity respectively). For simplicity, in the remainder of this paper, we consider CPTP1 and CPTP3 together and refer to them as CPTP1.

DISCUSSION

We have examined the expression of two PTPase genes in the developing rat CNS. By in situ hybridization we demonstrate that CPTP1 and

P19-PTP are expressed in the CNS by progenitor cells and by developing neurons. In addition, the analysis of a longer cDNA clone indicates that CPTP1 and CPTP3 represent the two phosphatase domains of a single PTPase gene.

CPTP1 gene encodes a novel receptor PTPase.

Recent PCR and low stringency hybridization studies have led to the identification of many novel PTPases (reviewed in (Fischer et al., 1991; Saito, 1993; Pot and Dixon, 1992; Charbonneau and Tonks, 1992). Within the family of PTPases, two subfamilies have been characterized: low molecular weight cytoplasmic (non-receptor) PTPases and high molecular weight transmembrane (receptor) PTPases. With the exception of two (HPTPβ and DPTP10D), all transmembrane phosphatases have two conserved intracellular phosphatase domains. The catalytic activity is associated with the domain proximal to the membrane while the second, more distal domain appears to have regulatory functions.

Both putative phosphatase domains of CPTP1 detect multiple high molecular weight transcripts, similar in size to receptor PTPases. In addition, they both show high sequence similarity to LAR and HPTPδ, two receptor PTPases. Thus, the sequence of and RNA transcripts detected by CPTP1 strongly suggest that it encodes a transmembrane PTPase. The in situ hybridization results presented here show that both domains of CPTP1 are expressed at very high levels in the developing brain. In particular, the ventricular zone of the cortical anlage at E15 and E17, where progenitor cells give rise to postmitotic neurons, is perhaps the highest region of CPTP1 expression. Expression is markedly down regulated in the adult, suggesting that these putative PTPases may have a role in the proliferation and early differentiation of neurons.

The putative phosphatase domains of CPTP1 are most similar to LAR and HPTPδ, two receptor-like PTPases that are highly similar to one another (63% in the extracellular region, 88% in the intracellular region). The extracellular domains of LAR and HPTPδ contain three Ig-like repeats and eight FN III-like repeats (Saito and Streuli, 1991). The combination of Ig- and FN III-like repeats is also found in cell adhesion molecules like NCAM (Rutishauser, 1983), fasciclin II, neuroglian (Grenningloh et al., 1990) and TAG-1 (Furley et al., 1990), as well as in a tumor suppressor gene product, DCC (Fearon et al., 1990). The high degree of similarity among the phosphatase domains of CPTP1, LAR and HPTPδ, leads to the prediction that the extracellular region of CPTP1 will also contain Ig- and FN III-like motifs. During the preparation of our report (Sahin and Hockfield, 1993), the full length sequence of CPTP1 was simultaneously identified from different tissues by four different groups, and so has also been named PTP NE-3 (Walton et al., 1993), PTP-P1 (Pan et al., 1993) and RPTP-σ (Yan et al., 1993), and LAR-PTP2 (Zhang et al., 1994). The full length sequence in fact does confirm the predicted structure of Ig- and FN III-like motifs. Such a structure might allow CPTP1 to play a dual role, cell adhesion and signal transduction, in neuronal differentiation in response to cell-cell or cell-matrix interactions.

CPTP1 may give rise to more than a single gene product.

On northern blots both putative phosphatase domains of CPTP1 recognize multiple transcripts, with two prominent bands at 7.8 and 6.5 kb. The presence of multiple bands may be due to processing of mRNA from a single gene, to the existence of multiple genes encoding the CPTPs, or to cross-hybridization to other mRNAs. Several lines of evidence support the first of these possibilities, i.e. the presence of differential splicing and/or

polyadenylation sites. First, on genomic Southern blots, carried out under high stringency conditions, CPTP1 hybridizes to a single gene. Second, it is not likely that the two bands represent cross-hybridization to other gene products, such as rat LAR, because the calculated melting temperature of CPTP1-LAR hybrids is lower than the stringency conditions used for the northern blots. In fact, all bands remain present when the stringency of the northern is increased by raising the wash temperature to 70°C. Taken together, these data indicate that the 6.5 and 7.8 kb CPTP1 transcripts represent two different mRNAs derived from a single gene, distinct from rat LAR.

Many receptor PTPases, including CD45 (Streuli et al., 1987), LAR (Zhang and Longo, 1995), LRP (Matthews et al., 1990), DPTP10D and DPTP99A (Yang et al., 1991; Tian et al., 1991), have variants with different splicing and polyadenylation sites. Alternative splicing of the CD45 gene is particularly interesting because it occurs in the region encoding the extracellular domain of the molecule. Eight isoforms of CD45 are produced using all possible combinations of the three exons encoding the extracellular domain (Trowbridge, 1991). These isoforms are expressed by different cell types and may have different ligands (Thomas, 1989). Therefore, receptor PTPases have the potential to generate a large number of cell type specific isoforms by alternative splicing.

Alternative splicing within the CPTP1 mRNA has been reported by two different groups. Screening different libraries, Goldstein and colleagues have identified a liver cDNA that contains eight FN III-like repeats and a brain cDNA that contains only five FN III-like repeats. Thus, CPTP1 gene may undergo tissue-specific alternative splicing leading to two different size transcripts. In an effort to account for the multiple bands seen on the northern blots of cortex, we looked for these extra FN III-like repeats in the P0 cortex by PCR, but have not

been able to detect their presence on ethidium bromide stained agarose gels (Daniel Wolf, M.S. and Susan Hockfield, unpublished observations). A more sensitive approach may be to do Southern blots with the deleted segments. In addition Stork and colleagues have shown that RNA processing in the 3' terminal of CPTP1 can lead to deletion of its second catalytic domain in tissues including the cortex. We do not detect this band in our northern blots and have not examined its distribution by PCR.

In summary, the identities of the multiple CPTP1 bands on the northern blots are not known at present. Our results raise the possibility that different isoforms of CPTP1 may be expressed by different groups of neural cells. Until we obtain additional sequence of these transcripts, we will not be able to resolve possible differential distributions of the mRNAs. However, the differential temporal regulation of the two major CPTP1 transcripts is consistent with this hypothesis. The expression of 7.8 kb and 6.5 kb transcripts is regulated differently during neural development. On northern blots, the 7.8 kb band is expressed at very high levels in the embryonic cortex while the 6.5 kb is not detected in the cortex until after birth. The in situ signal detected in the CNS of embryos therefore reflects the expression of the 7.8 kb message, while the signal detected in postnatal brain most likely reflects expression of both 7.8 and 6.5 kb messages. The differential regulation of the two CPTP1 mRNAs suggests that the two transcripts may encode gene products with different functions during pre- and post-natal brain development.

In addition to RNA processing, isoforms of receptor PTPases can be generated by post-translational modifications such as glycosylation and proteolytic processing. The receptor PTPases CD45, LAR and LRP are all heavily glycosylated (Thomas, 1989; Yu et al., 1992; Sap et al., 1990), and glycosylation of CD45 is critical for ligand binding (Stamenkovic et al., 1991).

The pattern of glycosylation of CD45 differs among different cell types and changes with activation of lymphocytes (Thomas, 1989). Differential glycosylation is also known to regulate the adhesive properties of other cell adhesion molecules, such as NCAM (Doherty et al., 1990), and thus may play a similar role for receptor PTPases. Furthermore, proteolytic processing of receptor PTPases may also be important in regulating their functions. Both human and rat LAR are cleaved intracellularly into two polypeptides that associate with each other non-covalently on the cell surface (Streuli et al., 1992; Yu et al., 1992). The extracellular 150 kd portion of LAR containing the Ig- and FN III-like repeats can be shed in cell culture (Streuli et al., 1992). Neither the mechanisms that regulate cleavage and shedding nor the effects of these phenomena on phosphatase activity are known.

P19-PTP is a non-receptor PTPase highly enriched in the embryonic CNS.

P19-PTP (CPTP2) recognizes a 3.5 kb message with sequence homology to non-receptor PTPases. Two other groups have isolated full length cDNAs of similar size from human colon (PTPG1; Takekawa et al., 1992) and from human skeletal muscle (PTP-PEST; Yang et al., 1993). By sequence homology, PTPG1 and PTP-PEST are the human homologs of the murine P19-PTP gene. In addition, two groups have recently reported PCR-generated clones with very high homology to our clone CPTP2. The deduced amino acid sequence of CPTP2 is identical to a sequence isolated from mouse myeloid leukemia cells (Yi et al., 1991) and is highly similar (one out of 107 amino acids different) to a sequence from rat kidney cDNA (Moriyama et al., 1992). Although the nucleic acid sequence was not reported, the molecular weight and tissue distribution of the mouse myeloid mRNA is similar to CPTP2. These sequence

data strongly suggest that all these cDNAs represent a single, ubiquitously expressed, non-receptor PTPase.

One major difference between the mouse myeloid PTPase and CPTP2 is that the level of the myeloid PTPase expression was reported to be equal in all tissues examined, including fetal and adult brain (Yi et al., 1991). This is in contrast to the down-regulation of P19-PTP mRNA we report here. The difference may be explained by the use of β-actin as a control for equal loading of RNAs probed with the myeloid PTPase probe. In the brain, actin isoforms are expressed at much higher levels during development than in the adult (Schmitt et al., 1977). An actin control may, then, have resulted in an underestimation of the mouse myeloid PTPase mRNA in the fetal brain.

Non-transmembrane PTPases have been isolated from the brain by other groups. T-cell PTPase (Cool et al., 1989), rat PTPase-1 (Guan et al., 1990) and STEP (striatum enriched phosphatase, Lombroso et al., 1991) are expressed in the brain. Rat PTPase-1 is found at high levels in adult hippocampus while STEP is primarily expressed in the adult striatum. The developmental expression profiles of these genes have not been reported. At present, P19-PTP is the only putative non-receptor PTPase which shows preferentially high levels of expression in embryonic brain. Our in situ hybridization results show that the P19-PTP is expressed at highest level in the developing cortex during the embryonic period, the time of rapid cellular proliferation. As development continues, the level of P19-PTP expression in the CNS decreases so that only a low level of expression can be detected in the adult cortex or spinal cord. P19-PTP expression in the adult liver is about the same as in the adult brain, much less than the embyonic brain.

Potential roles for CPTP1 and P19-PTP in the developing brain.

CPTP1 and P19-PTP both encode putative PTPases that are widely distributed in the developing CNS. Within the cortex, both P19-PTP and the 7.8 kb transcript of CPTP1 show very high levels of expression during the period of corticogenesis. Both are also expressed at high levels in the external granule cell layer of the developing cerebellum and are down-regulated throughout the nervous system in adult animals. Such temporal regulation of expression suggests that these PTPases may be involved in proliferation, migration and early differentiation of neurons. The 6.5 kb CPTP1 message is expressed at the same level throughout postnatal life, implying that it has a constitutive role in mature neural functions.

The role of intercellular interactions in normal physiological processes and during development is well established. With the identification of increasing numbers of cell-cell and cell-matrix adhesion molecules, greater insights into the molecular mechanisms underlying cellular interactions have been gained. For example, it has become increasingly clear that cell adhesion is not merely a matter of extracellular stickiness, but is a complex phenomenon involving transmembrane signaling and cytoplasmic responses. This has been demonstrated most clearly in the immune system. For instance, the binding of leukocytes to endothelium and of helper T cells to antigen presenting cells lead to intracellular reorganization mediated by second messengers (Kupfer and Singer, 1989; Springer, 1990; Butcher, 1991). Both events involve cell adhesion molecules of the Ig and integrin families. Similar signaling mediated by integrins is involved in adhesive functions of platelets (reviewed in Shattil and Brugge, 1991).

In the developing nervous system, the molecular mechanisms that underlie processes such as neuronal migration and axonal guidance are being studied in detail (Bixby and Harris, 1991; Hynes and Lander, 1992). While many

cell adhesion molecules have been shown to play a role in these processes, the signal transduction mechanism by which cell surface events are translated into cellular responses are poorly understood. Many cellular processes are regulated by protein phosphorylation, balanced by the competing activities of kinases and phosphatases. Cell-cell contacts and protein phosphorylation can reflect two components of a single process, as illustrated by genetic studies in Drosophila. Null mutations of either the cell adhesion molecule, *fasciclin I*, or a cytoplasmic PTK, *abl*, have little observable effect on the nervous system. However, embryos double mutant for *fasciclin I* and *abl* show a pronounced disorganization of the nervous system, presumably due to a developmental misrouting of growth cones (Elkins et al., 1990). The identification of two receptor PTPases expressed on subsets of growing axons in Drosophila further suggest that a PTPase might perform both cell-cell recognition and intracellular signaling functions during development (Yang et al., 1991; Tian et al., 1991); however, the test of such a hypothesis has not yet been reported.

In vitro studies of vertebrate neurons also suggest that tyrosine phosphorylation regulated by extracellular signals is involved in neurite outgrowth. Inhibition of PTKs by genistein facilitates substrate-induced neurite outgrowth (Bixby and Jhabvala, 1992). Furthermore, binding of soluble L1 and NCAM to growth cone membranes reduces tyrosine phosphorylation of tubulin (Atashi et al., 1992). Although the mechanism of the decreased phosphorylation is not known, PTPases may be involved by either directly dephosphorylating tubulin or by regulating the activity of the PTKs. PTPases such as CD45 and LRP have already been shown to regulate src-family PTKs (Sefton and Campbell, 1991; Zheng et al., 1992).

The distribution of the PTPases described here suggest a role in cell-cell communication early in neural development. In the developing cortex and

brainstem, CPTP1 and P19-PTP are expressed in many areas, but both are most abundant in the regions immediately surrounding the ventricles, which are the site of cellular proliferation. While the cells that occupy the proliferative zones of the embryonic brain are largely homogeneous in appearance, they give rise to all of the phenotypically diverse types of neurons and glia in the mature brain (for discussion, see Geschwind and Hockfield, 1989). The mechanisms that control cellular differentiation have been the object of intensive study over the last several years. Reports from two laboratories indicate that some aspects of the cell fate are determined as a consequence of signals transmitted and received during the terminal mitosis (Reh and Kljavin, 1989; McConnell and Kaznowski, 1991). The potential dual roles of receptor PTPases as cell adhesion and transmembrane signaling proteins would be well suited to mediate this kind of signal transduction.

Receptor PTPases such as CPTP1 and non-receptor PTPases such as P19-PTP are new candidates which may play adhesion and/or signaling roles mediating the accurate assembly of the nervous system. It might be safely predicted that the full repertoire of PTPases expressed in the brain has not yet been explored. While the sequences we described here are expressed quite abundantly in the neonatal cortex and throughout the developing nervous system, members of the PTPase family expressed in more specific temporal and spatial patterns probably remain to be discovered.

Figure 1. CPTP mRNA expression during development. Total RNA extracted from cortex on embryonic day 16 (E16) and postnatal days (P) 0, 4, 14, 30 and adult (AD) as well as P35 liver (LIV) were run in formaldehyde-agarose gels, blotted onto nitrocellulose. The same blot was used for four rounds of hybridization separately with ^{32}P-labeled cDNA probes for (A) CPTP1, (B) P19-PTP, (C) CPTP3, (D) control probe cyclophilin. The size of the transcripts were calculated using RNA molecular weight markers and are indicated at the left side of each blot. (E) Following exposure to the film, densitometry of the autoradiograms was performed for each transcript. To determine the relative abundance of each transcript in the RNA samples, the ratio of absorbance of each CPTP band to that of the cyclophilin band was calculated. In the graph, these values are presented as a percentage of the value in the adult cortex. According to this analysis, highest level of expression for P19-PTP and the 7.8 kb bands of CPTP1 and CPTP3 are found at E16. The 6.5 kb bands of CPTP1 and CPTP3 are detected at approximately equal levels in RNA samples from cortex at all postnatal ages, but are not detected in RNA samples from the E16 cortex or P35 liver.

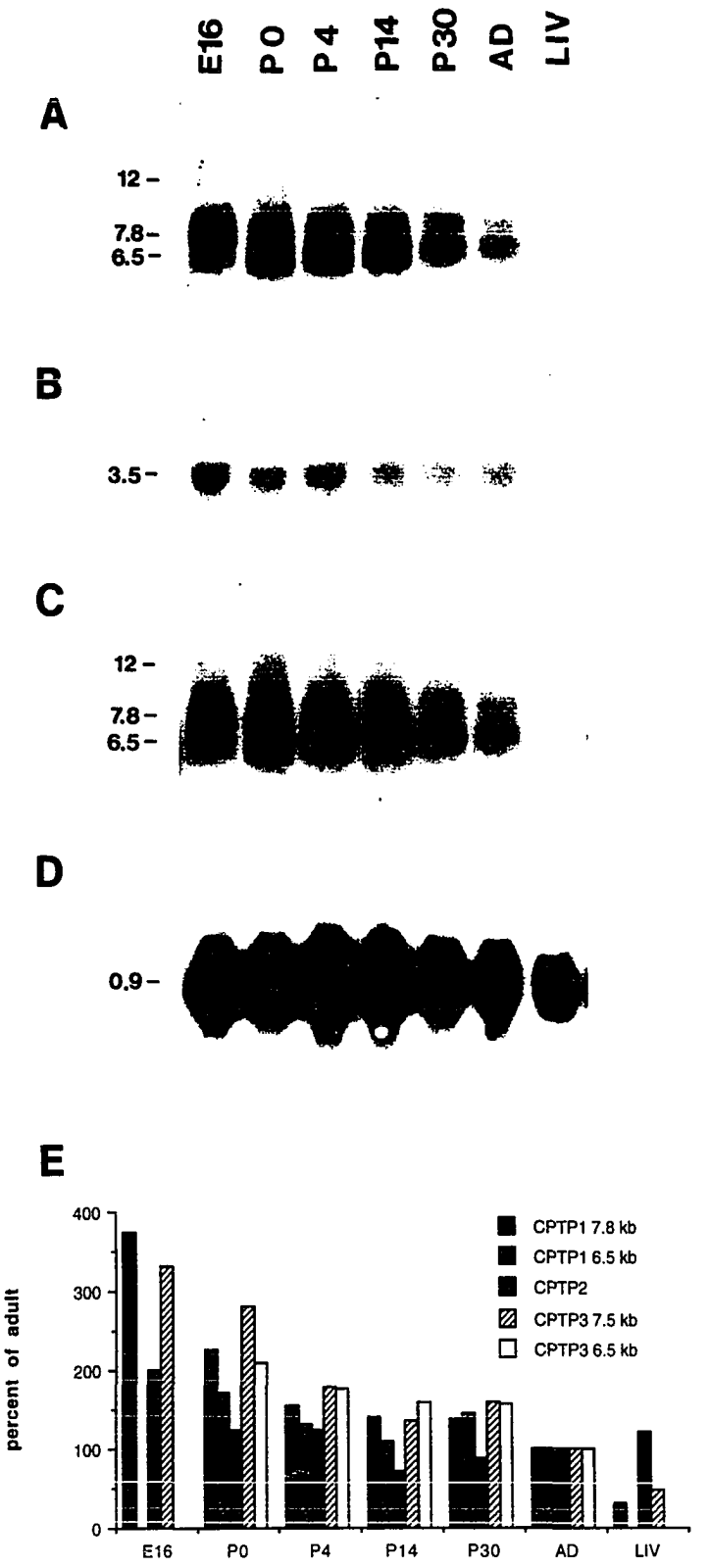


Figure 2. In situ hybridization demonstrates that CPTPs are expressed in the CNS during embryonic development. Parasagittal sections from embryonic day 15 (A-D) and 17 (E-H) animals were hybridized to ^{35}S-labeled antisense RNA probes for CPTP1 (A,E), P19-PTP (B,F), CPTP3 (C,G) and neurofilament-M (D,H) as control probe. **(A)** At E15, a high CPTP1 signal is detected in the cerebral cortex (c), midbrain (mb), medulla (m). **(B)** P19-PTP mRNA is present throughout the CNS including the spinal cord. **(C)** CPTP3 mRNA is expressed at high levels in the cerebral cortex and the midbrain. There is less but significant labeling in the medulla and the spinal cord. **(D)** NF-M signal is detected at the sacral end of the spinal cord, but only weakly in the medulla and at even lower levels in the telencephalon. **(E)** At E17, CPTP1 mRNA is found at highest levels within the cortex (c) surrounding the lateral ventricle (v) and in the midbrain (mb). Lower signal is detected in the medulla (m), spinal cord (s) and the DRGs (d). **(F)** In a near adjacent section, P19-PTP message is detected throughout the CNS and in the DRGs, with highest expression in the cortex. **(G)** At E17, CPTP3 signal is seen at high levels in the cortex and at somewhat lower levels in more caudal regions of the CNS. **(H)** NF-M signal is expressed at higher levels in the DRGs than the CNS; a weak signal is detectable in the spinal cord and medulla and an even weaker signal in the cortex. (Scale bar = 1mm)

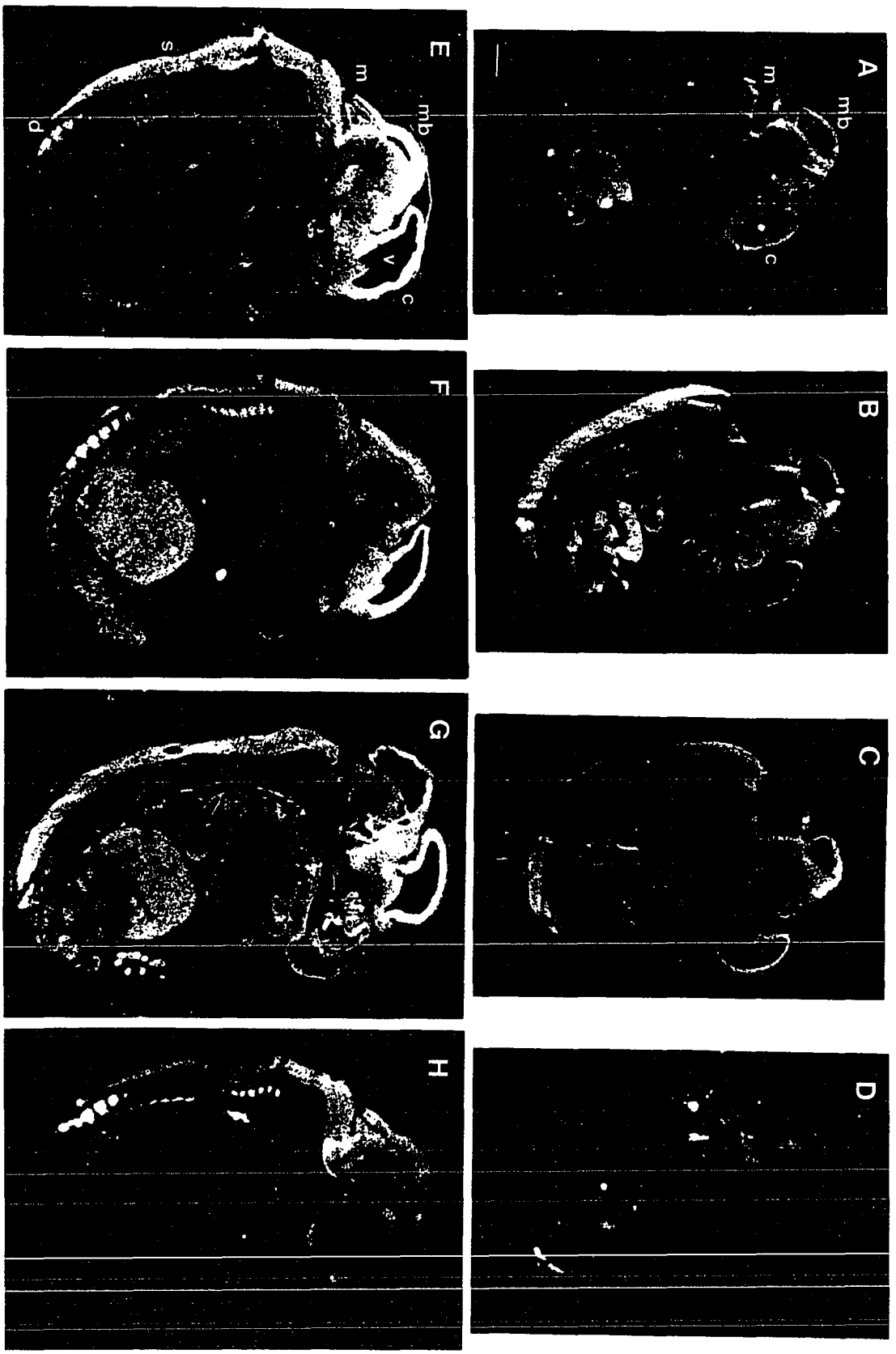


Figure 3. CPTP expression in postnatal brain. Parasagittal sections from postnatal day 4 (A-D) and adult (E-H) brains were hybridized to ^{35}S-labeled antisense RNA probes for CPTP1 (A,E), P19-PTP (B,F), CPTP3 (C,G) and NF-M (D,H) as control. **(A)** At P4, a high level of CPTP1 signal is detected in the neocortex (c), cerebellum (cb) and hippocampus (h). Within the neocortex, signal is especially high in the superficial layers and the subplate (arrow). In the cerebellar cortex, two bands of CPTP1 signal are detected, in the external and internal granular layers (see also Fig. 4D). In the hippocampus (h), all CA fields and the dentate gyrus exhibit high levels of CPTP1 hybridization. **(B)** P19-PTP signal is seen most prominently in the cerebellum while the rest of the brain displays diffuse hybridization. **(C)** CPTP3 mRNA is detected in a pattern very similar to CPTP1 (in A). Signal is most intense in the neocortex, hippcampus, dentate gyrus and cerebellar cortex. **(D)** NF-M is abundant in neocortex, hippocampus, thalamus and cerebellum. Within the neocortex, the highest signal is in the middle layers. In the cerebellum, the most superficial layer (external granular layer) shows less signal than the deeper layers. **(E)** In adults, the highest levels of CPTP1 mRNA is detected in the CA fields of hippocampus, dentate gyrus and entorhinal cortex. The neocortex displays only a diffuse signal. **(F)** P19-PTP message is detected at very low levels throughout the brain. **(G)** CPTP3 mRNA is expressed diffusely in the neocortex and hippocampal formation. **(H)** NF-M message in the adult is most prominent in the hippocampus and neocortex, but is detectable throughout the brain. (Scale bar = 1mm)

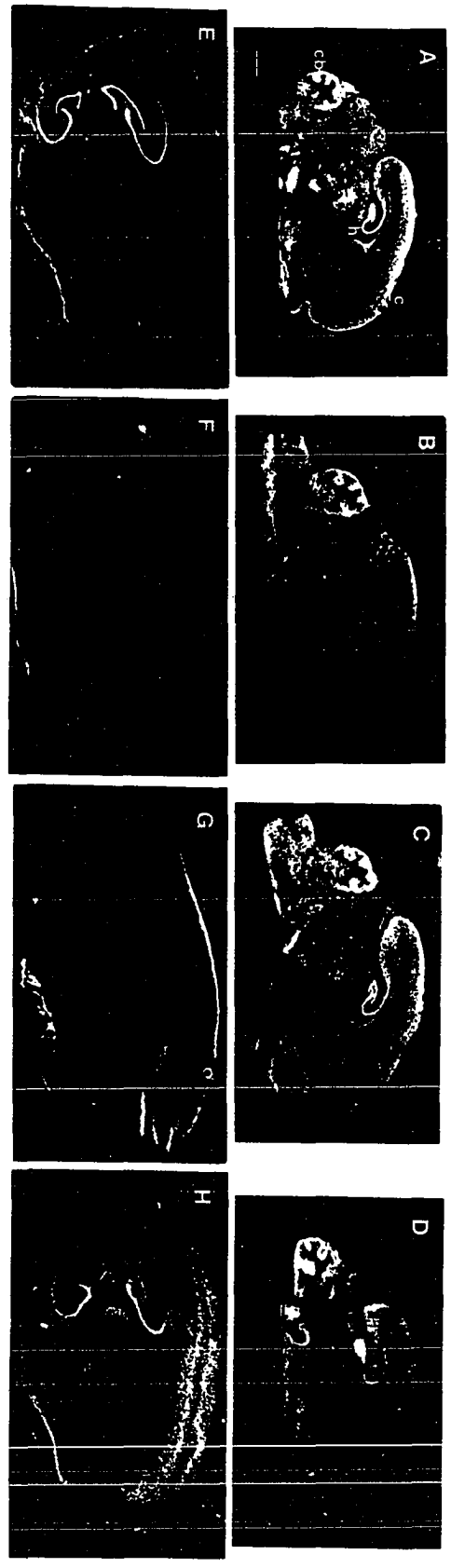


Figure 4. CPTP1 mRNA is detected in neurons. Emulsion dipped slides were counter stained with cresyl violet and photographed under bright-field optics for cell localization (A, C, E) or under dark-field optics for silver grain visualization (B, D, F). (A and B) Horizontal section through the neocortex on postnatal day 6 demonstrates CPTP1 mRNA in all layers of the cortex with highest levels in layer 2-3 and the subplate (s). Much less label is detected in the underlying white matter (wm). (C and D) CPTP1 mRNA is detected at high level in the external (single arrow) and internal (double arrow) granular layers of the cerebellum at postnatal day 4. Between the two granular layers, the Purkinje cell layer is relatively devoid of hybridization. (E and F) In the ventral horn of postnatal day 4 spinal cord, CPTP1 signal is very high over neurons in the gray matter (arrows). Note that hybridization in the neighboring white matter (wm) is much less intense. Scale bars for A-D = 200 μm; for E and F = 100 μm.

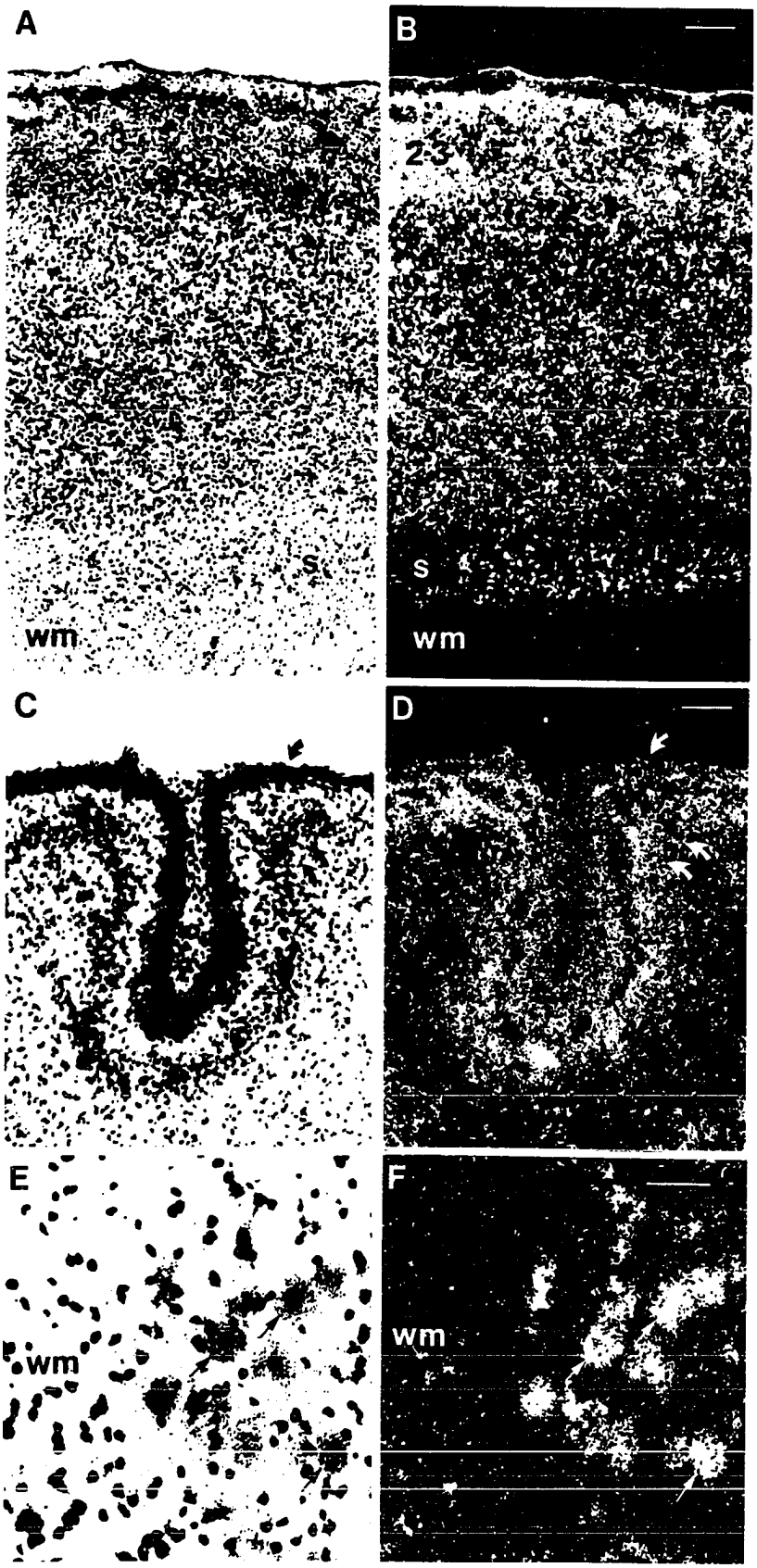


Figure 5. CPTP expression is restricted to gray matter in the spinal cord. Transverse sections from postnatal day 4 (A-D) and adult (E-H) spinal cord were hybridized to ^{35}S-labeled antisense RNA probes for CPTP1 (A,E), P19-PTP (B,F), CPTP3 (C,G) and NF-M (D,H) as control probe. At both ages, the sections for CPTP1 and NF-M are taken from cervical segments while those for P19-PTP and CPTP3 are from thoracic segments. **(A)** CPTP1 is expressed throughout the spinal gray matter, with somewhat higher levels of expression in the spinal dorsal horn. Expression of P19-PTP **(B)**, CPTP3 **(C)** and NF-M **(D)** are similarly restricted to the gray matter. NF-M (D) is expressed at higher levels in the ventral horn. In the adult, CPTP-1 **(E)**, P19-PTP **(F)** and CPTP3 **(G)** expression is diminished, but still greater in gray than in white matter. **(H)** At this stage, NF-M is highly expressed throughout the spinal gray matter. (Scale bar = 1mm)

CHAPTER 3

A PROTEIN TYROSINE PHOSPHATASE, PTPH1, IS EXPRESSED IN A SEGMENT-SPECIFIC PATTERN IN THE RAT DIENCEPHALON

SUMMARY

PTPases that we have reported in the previous chapters are widely distributed within the rat brain. Here, we show that a non-receptor PTPase, PTPH1, is specifically expressed in the adult thalamus. Within the thalamus, PTPH1 expression is detected in most, but not all nuclei. Nuclei derived embryonically from the dorsal thalamus express this gene while those derived from the ventral thalamus do not. During development, PTPH1 expression is also greatly restricted to the dorsal thalamus as early as embryonic day 19. Taken together, these observation suggest that PTPH1 is a specific marker for the developing and mature dorsal thalamic nuclei.

INTRODUCTION

The mature brain of all species can be divided into different regions on the basis of distinct cellular and biochemical composition, patterns of connectivity and electrophysiological properties. While the regional organization of the mammalian brain is well established, the molecular mechanisms by which regional differences develop are largely unknown. One approach to this problem is to identify genes that are expressed in different regions.

The search for segment-specific genes has been successful first in the hindbrain and more recently in the forebrain. Studies of the hindbrain rhombomeres, a series of segmental bulges that give rise to brainstem structures (Hunt and Krumlauf, 1991), have revealed a number of genes that are expressed in rhombomere-specific patterns. For example, the expression of the putative transcription factor, Hox-b1, is restricted to rhombomere 4 (r4) (Wilkinson et al., 1989). The flanking rhombomeres, r3 and r5, express Krox-20, a transcription factor of the zinc-finger class. The forebrain also can be divided into a number of transverse segments based on morphology as well as expression patterns of regulatory genes (Rubenstein et al., 1994). For example, the diencephalon is made up of three segments named p1, p2 and p3 which respectively give rise to the pretectum, dorsal thalamus and ventral thalamus. Homeodomain containing proteins such as Dlx-1, Dlx-2 and Gbx-2 act as markers for each of these domains (Bulfone et al., 1993).

In addition to transcription factors, another group of genes likely to play a role in regional differentiation are those encoding proteins involved in intercellular signaling. In fact, four receptor protein tyrosine kinases (PTKs) that are expressed in rhombomere-specific patterns have been identified (Nieto et

al., 1992; Henkemeyer et al., 1994; Becker et al., 1994). All four genes, named "segmentally expressed kinase" (Sek) 1-4, belong to the Eph subfamily of receptor PTKs, and each has an overlapping, but unique expression pattern in the hindbrain. This rhombomere specificity suggests that tyrosine phosphorylation may play a role in the regional specification of the mammalian brain.

Since the state of tyrosine phosphorylation is determined by the opposing actions of PTKs and protein tyrosine phosphatases (PTPases), one might expect PTPases also to be involved in regional specialization. Indeed, there is growing evidence that PTPases may play significant roles in regional differentiation. In Drosophila, three receptor PTPase genes are expressed by subsets of neurons during axonogenesis (Yang et al., 1991; Tian et al., 1991; Hariharan et al., 1991). Two of these, DLAR and DPTP99A, are relatively uniformly expressed in all of the developing connectives and commissures, while the third, DPTP10D, is enriched in the longitudinal connectives and anterior commissures. Similar to the Drosophila PTPases, a mammalian non-receptor PTPase is predominantly expressed in the striatum and is designated "striatum enriched phosphatase" (STEP) (Lombroso et al., 1991). The gene that gives rise to STEP has at least two alternatively spliced forms. 4.4 kb message is present in many brain areas while 3.0 kb band is particularly enriched in the striatum.

Given the possible involvement of PTPases in neural development, we have investigated the expression of PTPase genes in the developing mammalian CNS (Sahin and Hockfield, 1993; Sahin et al., 1995). In this paper, we report that a non-receptor PTPase, PTPH1, is expressed in a regionally specific pattern in the rat brain. PTPH1 was originally cloned from HeLa cells and has homology to the cytoskeleton-associated proteins band 4.1, ezrin and

talin (Yang and Tonks, 1991). Here, we show that PTPH1 expression is markedly enriched in the thalamus in the adult rat brain. Within the thalamus, PTPH1 mRNA is expressed in most, but not all nuclei. PTPH1 is not expressed in nuclei that are embryonically derived from the ventral thalamus. During development, PTPH1 gene expression is specific to the dorsal thalamus as early as E19 and maintains this regional specificity throughout postnatal ages. These observations suggest that PTPH1 is an early and specific marker for the dorsal thalamus and may play a role in its differentiation.

MATERIALS AND METHODS

Northern Hybridization

For the analysis of tissue distribution, northern blots of RNA from various regions of adult Sprague-Dawley rat brains were generously supplied by Tony Ciaberra, Yale University. Total RNA was extracted from rat brain using RNAzol B (CINNA/BIOTECX), based on the method of Chomczynski and Sacchi (1987). Total RNA (20 µg/lane) was separated on a 1% agarose-formaldehyde gel and transferred to Biotrans(+) nylon membrane (ICN). Equality of loading levels was confirmed by adding ethidium bromide to each RNA sample and examining the 28S and 18S rRNAs under UV illumination. Northern hybridization was carried out in 7% SDS, 1% BSA, 0.5 M phosphate buffer pH 6.8 (PB), 1 mM EDTA for 18 hours at 65°C. Hybridization solution contained 1.2 x 10^6 cpm/ml of probe made by random primed labeling. After hybridization, the blot was washed twice in 5% SDS, 0.5% BSA, 40 mM PB, 1 mM EDTA and once in 1% SDS, 40 mM PB, 1 mM EDTA at 65°C for 15 minutes each. RNA molecular weight standards (GIBCO/BRL) were ran on the blot to estimate the sizes of the transcripts. The blot was exposed to Amersham Hyperfilm between two intensifying screens for 7 days.

In situ hybridization

In situ hybridization was performed as previously described (Sahin et al., 1995). Twelve micron thick frozen sections were thaw-mounted onto gelatin coated slides, post-fixed in 4% paraformaldehyde (pH 7.4) and acetylated with 0.5% acetic anhydride in 0.1 M triethanolamine (pH 8.0). They were then dehydrated in ethanols and delipidated in chloroform. Sections were prehybridized in 2X SSC, 50% formamide at 50°C for 1 hr, and then hybridized in 50% formamide, 1X Denhardt's, 0.75 M NaCl, 10% dextran sulfate, 30 mM DTT, 10 mM Tris-HCl pH 7.5, 1 mM EDTA, 0.5 mg/ml tRNA, 100 μg/ml salmon sperm DNA and 1 $x10^6$ cpm probe at 50°C for 12-15 hours. The [^{35}S]-CTP (NEN) labeled cRNA probes were generated using the T7 or T3 promoters in the Bluescript vector and the Riboprobe system (Promega). Following hybridization, slides were washed in 2X SSC, 50% formamide, 0.1% BME (β-mercaptoethanol) at 50°C, treated with 20 μg/ml RNase A, washed in 2X SSC, 50% formamide, 0.1% BME at 58°C and in 0.1X SSC/0.1% BME at 65°C for 30 min each.

For initial localization of the probe, slides were exposed to film (Hyperfilm, Amersham) for 3-14 days. Autoradiograms were used as negatives for prints. For higher resolution analysis of probe distribution, slides were dipped in NTB-2 emulsion (Kodak), developed after 35 days and counter-stained with cresyl violet. RPTPγ antisense (Sahin et al., 1995) and PTPH1 sense probes were used as positive and negative controls, respectively.

RESULTS

PTPH1 has a single transcript in the adult rat brain.

While conducting in a chromosome-walk on chromosome 9, several fragments of the PTPH1 gene were identified (Church et al., 1993). These

fragments were cloned in Bluescript plasmid vectors and used to determine the tissue distribution of PTPH1 RNA. Clone D3 (bps 1337-1586) was chosen for this analysis because it lies in a portion of PTPH1 cDNA which does not show homology to other genes in the GenBank.

The expression of PTPH1 in different regions of the adult rat brain was determined initially by northern hybridization (Fig. 1). Hybridization with clone D3 reveals a single band of 4.4 kb, which is very close in size to the transcript detected in HeLa cell mRNA (Yang and Tonks, 1991). This band is detected only in the lane containing RNA from the adult thalamus. The RNA from other regions of the adult brain including amygdala, brainstem, cerebellum, cortex, hippocampus, hypothalamus, olfactory bulb, spinal cord and striatum do not produce a signal at an exposure time sufficient to give a strong signal in thalamus RNA. After much longer exposure times (30 days), very faint bands of the same molecular weight are detectable in the RNA from hippocampus and cerebellum (data not shown). These data indicate that D3 is a specific probe for PTPH1 gene on northern hybridization and suggest that PTPH1 expression is greatly enriched in the rat thalamus.

PTPH1 expression in the adult thalamus

To confirm the thalamus-enriched expression of PTPH1, in situ hybridization studies were performed using adult brain sections. Parasagittal sections of the whole brain were hybridized with antisense D3 probe. The labeling is most intense in the thalamus (Fig. 2A). Within the thalamus, the labeling is not uniform and is most intense in certain nuclei, including the medial geniculate nucleus, the lateral geniculate nucleus and the ventral basal complex. The hippocampus and cerebellum also show hybridization signal, but at much lower levels. As a control, a probe for RPTPγ, a receptor PTPase, was

used (Barnea et al., 1993; Sahin et al., 1995). This phosphatase was chosen because it is expressed in the thalamus during early postnatal ages (Sahin et al., 1995). Hybridization with this receptor PTPase is highest in the adult cortex, especially in layer 4, as well as in the hippocampus (Fig. 2B). A much lower level of signal is seen in the adult thalamus. Hybridization with the sense probe for D3 produces no signal (Fig. 2C). These experiments show that the hybridization of D3 to the thalamus is specific and is different from other PTPases, which are more widely expressed in the brain.

Given that the distribution of labeling within the thalamus was not uniform in sagittal sections, a series of coronal sections of the thalamus were studied to identify the specific nuclei that contained PTPH1 RNA. To distinguish among the thalamic nuclei, emulsion dipped slides were counter stained with cresyl violet. Autoradiograms and high magnification micrographs are shown in Fig. 3 and the results are summarized in Table 1. In the most anterior sections examined (Fig. 3A), PTPH1 is expressed in the ventral medial, ventral posterior (VP), central median, mediodorsal and lateral dorsal thalamic nuclei. Labeling is absent from the reticular thalamic nuclei, zona incerta, subthalamus and habenula. In more posterior sections (Fig. 3B), PTPH1 labeling is again detected in the VP thalamic nuclei as well as in the dorsal lateral geniculate (DLG) and posterior (Po) thalamic nuclei. The superior thalamic radiation accounts for the gap between the VP and DLG nuclei. At this level, the ventral lateral geniculate (VLG) nuclei and zona incerta also show no labeling (Figs. 3D and E). Moving further posteriorly in the thalamus (Fig. 3C), the medial geniculate nuclei are the only are labeled diencephalic structures. Both ventral and dorsal medial geniculate (MG) nuclei contain PTPH1 labeling. At this level, the pretectum and superior colliculi do not show any PTPH1 signal. In all these sections, pyramidal cells in fields CA 1-3 of the hippocampus and granular cells

of the dentate gyrus show hybridization. Adjacent sections probed with antisense RPTPγ and sense PTPH1 probe exhibited no signal in the diencephelon (data not shown).

PTPH1 is restricted to the dorsal thalamus during development.

We next examined PTPH1 expression during development by in situ hybridization with sections of brain starting at embryonic day 19 (E19). PTPH1 RNA was detectable as early as E19 in the diencephalon in horizontal sections of the head (Fig. 4A). At this stage, hybridization is seen in the anlage of the dorsal thalamus and is absent from ventral thalamus (Figs. 4D and E; also see Jones, 1985). In addition to the diencephalic labeling, PTPH1 mRNA is also detectable at high levels in the olfactory epithelium at E19, but at very low levels in the neocortex and hippocampus. PTPH1 is also expressed in the dorsal root ganglia as early as E17 (Fig. 5). High levels of expression in the olfactory epithelium is consistent with the results of a previous PCR study that identified PTPH1 cDNA in the olfactory epithelium (Walton et al., 1993). By postnatal day eight (P8), PTPH1 signal is most intense in the thalamus compared to the rest of the brain (Fig. 4B). Neocortex and hippocampus express PTPH1 mRNA at low levels throughout the postnatal period. The thalamic-enriched expression of PTPH1 persists during the first two weeks of development (Fig. 4C). In summary, PTPH1 is expressed in a thalamus-enriched pattern throughout postnatal life.

DISCUSSSION

In this study, the expression pattern of a non-receptor PTPase, PTPH1, has been characterized. PTPH1 is highly enriched in the adult thalamus compared to other parts of the brain. Within the thalamus, the expression of

PTPH1 is widespread, excluding only the ventral lateral geniculate and reticular thalamic nuclei. The thalamus-enriched pattern of expression is established as early as E19 and is retained throughout postnatal development. Taken together, these observations indicate that, like STEP, PTPH1 is a non-receptor PTPase that is expressed in a region-specific pattern in the mammalian brain.

Non-receptor PTPases have been isolated from the brain by other groups. PTPase-1 (Guan et al., 1990), syp (Adachi et al., 1992; Feng et al., 1993; Ahmad et al., 1993) and STEP (Lombroso et al., 1991) are expressed in the brain. Previous studies have shown that syp, PTP1B and P19-PTP are widely expressed throughout the developing CNS (Feng et al., 1993; Sahin et al., 1995). In contrast, STEP and PTPH1 expression is restricted to the striatum and thalamus, respectively.

PTPH1 expression during segmentation of the diencephelon

Morphologically, the diencephelon can be divided into four segments: epithalamus, dorsal thalamus, ventral thalamus and hypothalamus (Coggeshall, 1964). Recently, three lines of evidence have demonstrated that the diencephelon is a segmented structure composed of several neuromeres similar to those in the hindbrain. First, clones of cell generated from a single precursor are restricted to one neuromere (Figdor and Stern, 1993). Second, adjacent groups of cells in different neuromeres do not mix (Figdor and Stern, 1993). Finally, the expression patterns of certain regulatory genes respect neuromeric borders. For instance, Dlx-1 and Dlx-2 are preferentially expressed in the ventral thalamus during embryonic development while Wnt-3 and Gbx-2 are expressed in the dorsal thalamus (Salinas and Nusse, 1992; Bulfone et al., 1993). The diencephalic neuromeres thus identified have been designated as D1 through D4 by Figdor and Stern (Figdor and Stern, 1993) or P1 through P3

by Rubenstein and Puelles (Rubenstein et al., 1994). In this discussion, we will use the numbering system from the Rubenstein and Puelles neuromeric model.

PTPH1 is the first PTPase gene reported to be expressed in a segment-specific pattern in the diencephalon. Its expression in the adult thalamus includes all thalamic nuclei except for the ventral LGN and reticular nucleus. These two nuclei differ from the rest of the thalamus in two major ways: both are embryonically derived from the ventral thalamus and neither projects to the cortex (Jones, 1985). The other nuclei that make up the adult thalamus are derived from the embryonic dorsal thalamus and almost all have thalamocortical connections. Therefore, PTPH1 is expressed in all adult thalamic nuclei generated by the dorsal thalamus and is absent in the nuclei generated from the ventral thalamus. Furthermore, PTPH1 mRNA is not detected in the zona limitans which acts as the border between the dorsal and ventral thalami. The expression pattern of PTPH1 is most consistent with the location of the diencephalic neuromere P2. Although P2 contains both the dorsal thalamus and the epithalamus, PTPH1 is absent in the epithalamus and habenula, and thus is restricted to a subsegment of P2.

The two other genes that are restricted to the P2 segment of the diencephalon are Wnt-3 and Gbx-2. Wnt-3 belongs to a family of secreted glycoproteins that are required for pattern formation in Drosophila and mammalian embryogenesis. For example, transgenic mice with null mutations of Wnt-1 gene lack a midbrain (McMahon and Bradley, 1990). Gbx-2 on the other hand is a transcription factor that belongs to the homeobox family (Murtha et al., 1991; Bulfone et al., 1993). While Wnt-3 is expressed in the epithalamus in addition to the dorsal thalamus, Gbx-2 and PTPH1 are restricted to the dorsal thalamus. Thus, PTPH1 expression overlaps more closely with the homeobox gene Gbx-2 than with Wnt-3.

While PTPases have been shown to be expressed in region-specific patterns in the Drosophila and in the mammalian brain, there are differences between their expression in the two systems. First, unlike STEP and PTPH1, axon-pathway specific PTPases in the Drosophila are receptor PTPases. Second, subsequent to axonogenesis, the mRNAs for the Drosophila PTPases are down-regulated, strongly suggesting that they play a role in establishing, rather than maintaining, axon pathways. However, expression of STEP and PTPH1 are maintained in the adult in a region-specific manner. Like PTPH1, Wnt-3 expression is also retained within the adult thalamus (Salinas and Nusse, 1992). The co-expression of Wnt-3 and PTPH1 in the adult thalamus suggests that these genes may also be involved in the maintenance of mature neural phenotype or function. Since the Wnt family of glycoproteins (Hinck et al., 1994) and tyrosine phosphorylation (Behrens et al., 1994) both can regulate cell-cell adhesion by modulating cadherin function, it is tempting speculate that PTPH1 may be a part of the effector pathway for Wnt-3 in the dorsal thalamus. In this hypothetical model, the N-terminus of PTPH1, which shows homology to cytoskeletal proteins, might allow it to act on other cytoskeleton-associated proteins such as catenins, thus modulating cell adhesion and morphology.

In summary, this is the first report of a PTPase that is expressed in a segment-specific pattern in the diencephalon. The expression pattern of PTPH1 demonstrated in this study provides further support for the neuromeric theories of forebrain development and suggests that PTPases may play a role in the regional differentiation of the nervous system.

Table 1: Expression of PTPH1 mRNA in diencephalic nuclei

NUCLEUS	PTPH1 RNA
Dorsal Thalamus	
anterodorsal n.	+
anteroventral n.	+
anteromedial n.	+
ventral medial n.	+
ventral posterior n.	+
central median n.	+
mediodorsal n.	+
lateral dorsal n.	+
dorsal lateral geniculate n.	+
posterior n.	+
dorsal medial geniculate n.	+
ventral medial geniculate n.	+
Ventral Thalamus	
reticular n.	-
ventral lateral geniculate n.	-
Zona Inserta	-
Epithalamus	
Habenula	-

Figure 1. Northern hybridization demonstrates that PTPH1 mRNA is particularly enriched in the thalamus. Total RNA extracted from different regions of the adult central nervous system is hybridized with probe D3. Only the lane containing RNA from the thalamus shows a band of 4.4 kb (top panel). Equal loading of the lanes is confirmed by ethidium bromide staining and photographing the gel. The rRNA visualized in this manner is shown in the bottom panel.

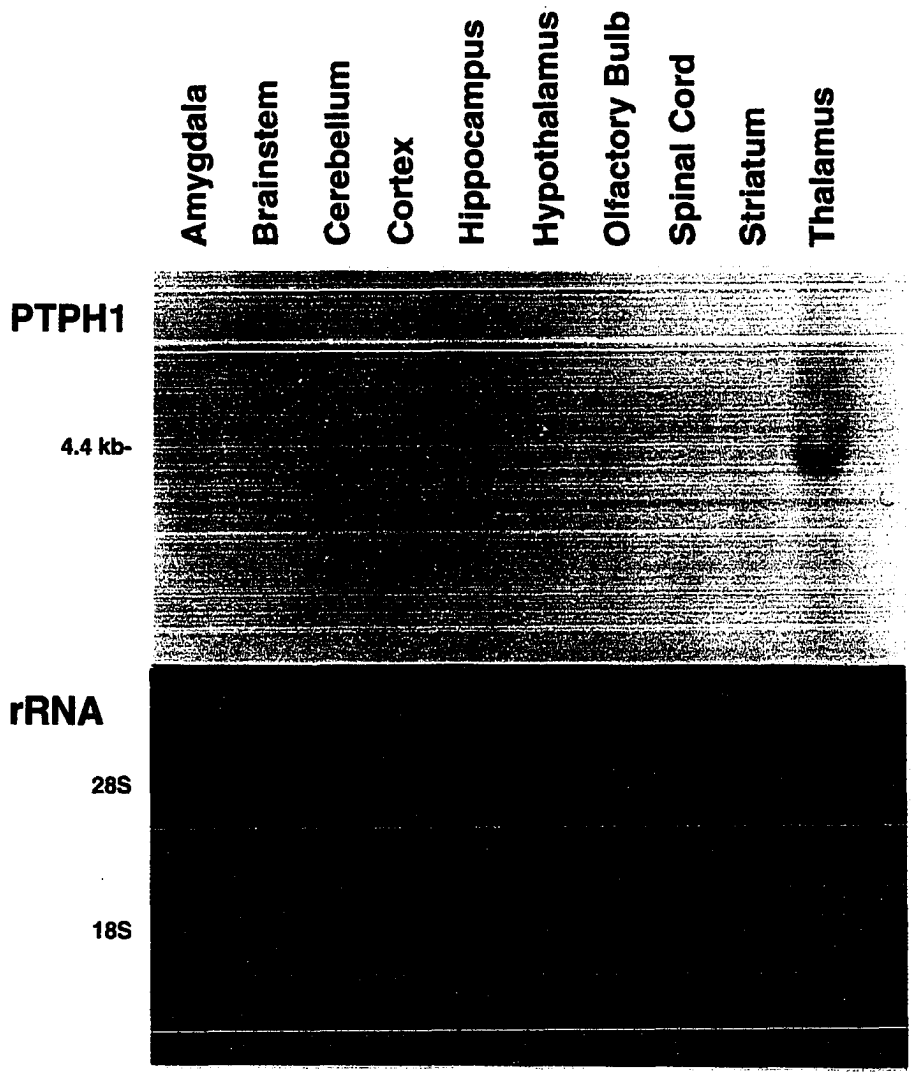


Figure 2. In situ hybridization demonstrates that PTPH1 mRNA is largely restricted to the thalamus. Near adjacent, parasagittal sections of adult brains were hybridized with radiolabeled antisense RNA probes for PTPH1 (A) and RPTPγ (B) and sense probe for PTPH1 as a negative control (C). (A) PTPH1 RNA is greatly enriched in the thalamus (t). Low levels of signal are seen in the hippocampus (h) and cerebellum (cb). (B) RPTPγ RNA expression is highest in the hippocampus, and is also present in the neocortex (ctx), particularly in the middle layers (arrow), and in the cerebellum. No signal is seen in the thalamus. (C) Sense probe for PTPH1 shows no signal in the brain. (Scale bar = 100 μm)

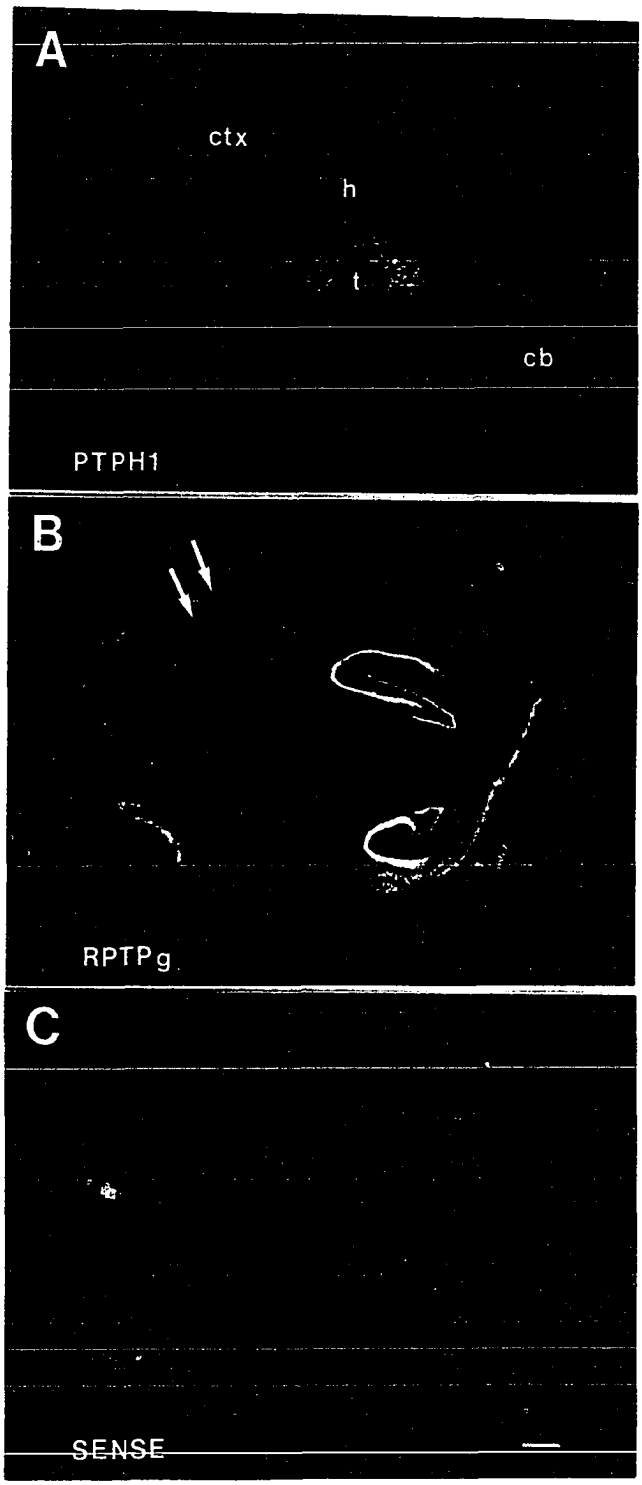


Figure 3. PTPH1 is expressed in most, but not all, thalamic nuclei. Serial coronal sections of the adult thalamus were hybridized with radiolabeled antisense PTPH1 probe. (A) PTPH1 is detected in the ventral medial (VM), ventral posterior (VP), central median (CM), mediodorsal (MD) and lateral dorsal (LD) thalamic nuclei. Labeling is absent from the reticular thalamic nuclei (R) and habenula (ha). (B) PTPH1 is expressed in the ventral posterior (VP) as well as in the dorsal lateral geniculate (dLG) and posterior (Po) thalamic nuclei. (C) In more caudal sections, the medial geniculate nuclei (MG) are intensely labeled.The superior colliculi (sc) do not show any PTPH1 signal. In all these sections, CA fields of the hippocampus (CA) and the dentate gyrus show low hybridization signal. (D and E) Emulsion dipped slide of a section near adjacent to (B) was counter stained with cresyl violet and photographed under bright-field optics for cell localization (D) and under dark-field optics for visualization of the silver grains generated by PTPH1 probe (E). PTPH1 signal is very high over neurons (arrows) in dorsal lateral geniculate (dLG) nucleus. In contrast, neurons in the neighboring ventral lateral geniculate (vLG) nucleus contain no labeling. White matter (wm) similarly shows no labeling (Scale bar for A-C = 1.5 mm; for D-E = 25 μm).

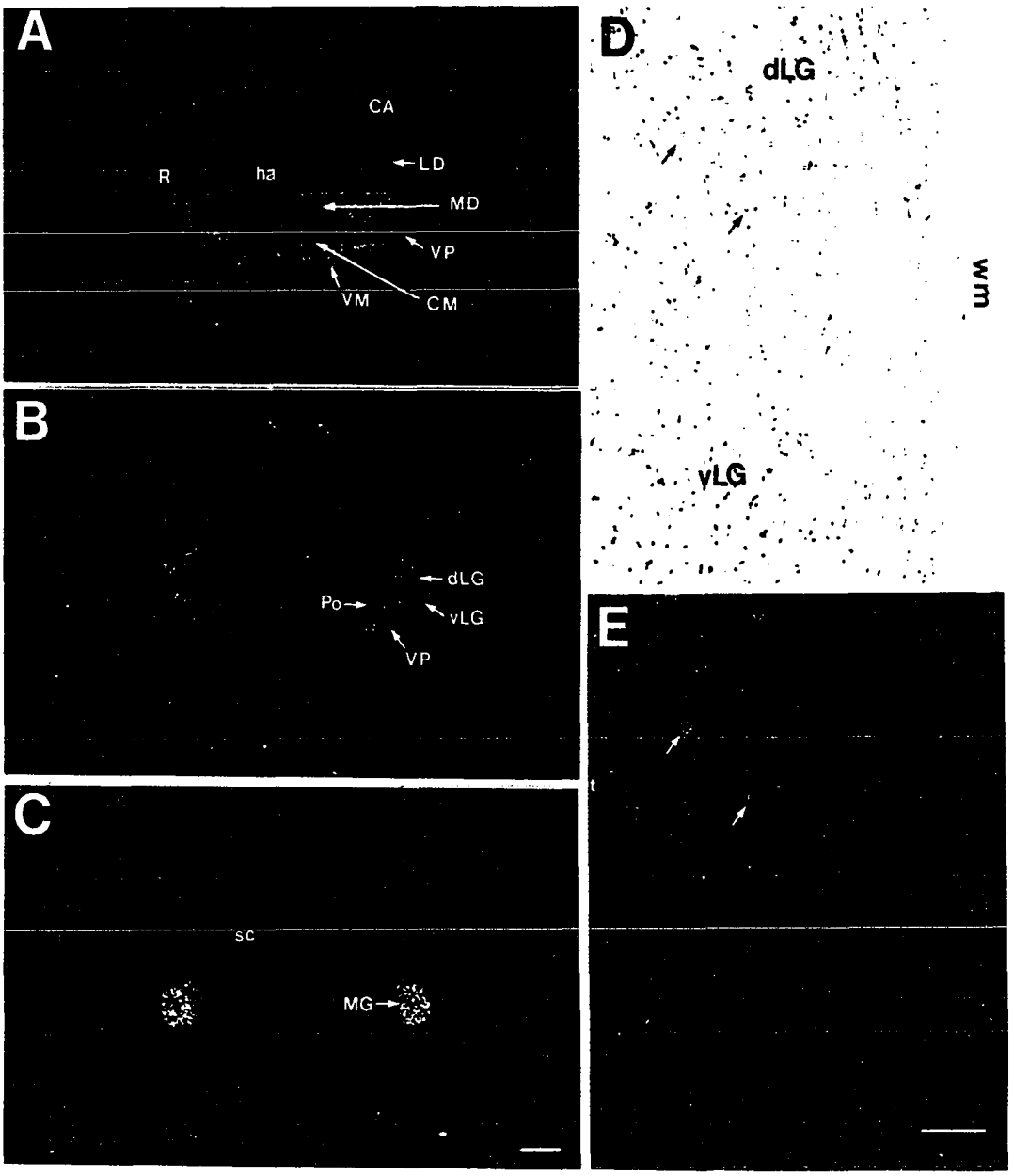
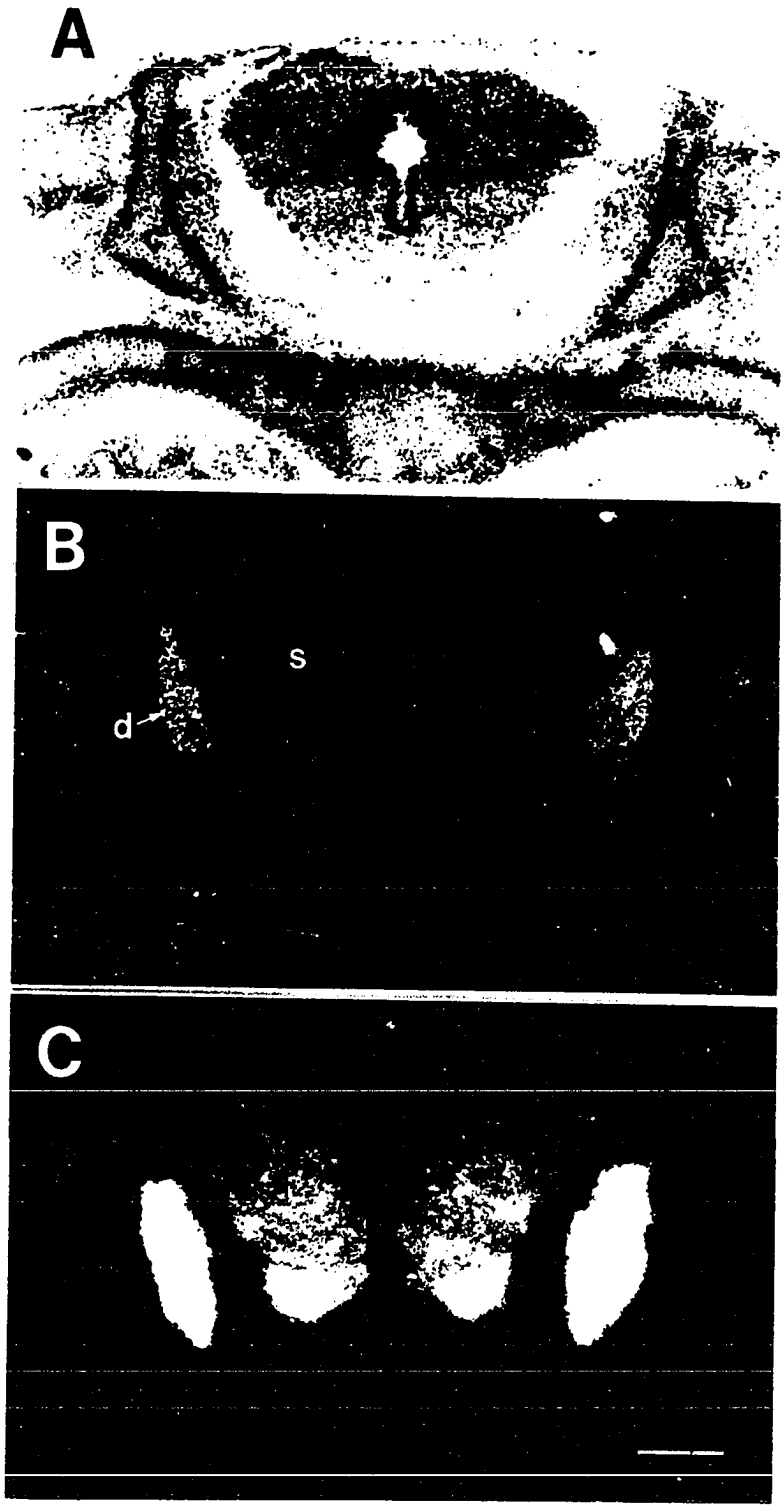


Figure 4. PTPH1 expression is restricted to the dorsal thalamus throughout development. Transverse section of E19 head (A) and coronal section of P8 (B), P15 (C) and adult (D) brains are hybridized with the antisense probe for PTPH1. (A) Within the E19 brain, the diencephalon (d) shows labeling for PTPH1 RNA. A much lower level of signal is detected in the cortex (c). Outside of the brain, the olfactory epithelium (oe) also exhibits intense PTPH1 signal. There is no labeling in the retina (r). (B) At P8, PTPH1 mRNA is detected in the thalamus (t) and hippocampus. (C) By P15, PTPH1 labeling is most intense in the thalamus. The hippocampus and entorhinal cortex show low levels of labeling. (D and E) Emulsion dipped and counter stained slide of a section near adjacent to that in (A) was photographed under dark-field illumination (D) for PTPH1 silver grain visualization and under bright field illumination (E) for cellular localization. PTPH1 labeling is detected in the dorsal thalamus (d), but not in the ventral thalamus (v). Anterior commissure (a) and third ventricle (3) are shown for orientation (Scale bars for A = 100 μm; for B-C = 100 μm; for D-E= 40 μm)

Figure 5. PTPH1 is expressed in the embryonic dorsal root ganglia. Emulsion dipped slides were counterstained with cresyl violet and photographed under brightfield optics for cell localization (A) and under darkfield optics for visualization of silver grains generated by probes to PTPH1 (B) and NF-M (C). (A) Transverse section through the lumber spinal cord at E17 shows the spinal cord, dorsal root ganglia and the cartilaginous anlage of the vertebra. The dorsal surface of the embryo is on the top. (B) Same section under dark field optics demonstrates that PTPH1 mRNA is detected in the dorsal root ganglia at high levels. Very little label is found in the spinal cord or in the cartilage. (C) A near adjacent section labeled with antisense NF-M probe shows that NF mRNA is localized to the dorsal root ganglia as well as the spinal cord. Ventral horns contain more labeling than the dorsal horns. (Scale bar = 25 μm)



GENERAL DISCUSSION

Although the first PTPase was cloned only six years ago, the field of PTPases has flourished rapidly. While the research for my dissertation was in progress, knowledge about PTPase expression in different systems has expanded at a striking pace. The number of PTPase genes that have been identified to date may not be 1002 as some recent review titles suggest (Charbonneau and Tonks, 1992; Pot and Dixon, 1992), but is over 25 not counting the species homologs. Furthermore, different PTPase genes are known to to play a role in processes as diverse as cell morphology in Dictyostelium (Howard et al., 1993) to axon growth in Drosophila (Yang et al., 1991; Tian et al., 1991; Hariharan et al., 1991), and ion channel regulation in Aplysia (Wilson and Kaczmarek, 1993) to hematopoesis in mammals (Shultz et al., 1993).

In this dissertation, I have reported the expression pattern of seven PTPases that are broadly distributed in the neonatal rat neocortex as well as one PTPase that is restricted to the dorsal thalamus. This chapter summarizes what we know about the structure and function of each of the PTPase genes expressed in the mammalian CNS. Six receptor and six non-receptor PTPases will be discussed below. This will be followed by description of a biochemical model of neurite outgrowth in which a putative role for PTPases is proposed. Finally, future prospects in this field will be reviewed with special focus on new experimental approaches.

RECEPTOR PTPases IN THE CNS:

Motivated by the identification of pathway-specific receptor PTPases in the Drosophila, several groups have sought to characterize receptor PTPases

expressed in the mammalian brain. To date, these studies have identified at least six receptor PTPases, which fall into three groups based on the structure of their extracellular domains (Fig. 1). The first group includes LAR, CPTP1 and PTPδ, all of which contain Ig- and FN III-like repeats. The second group consists of RPTPγ and PTPζ, which have carbonic anhydrase-like domains at the N-termini of their extracellular domains. The third group contains LRP, which has a very short extracellular region with no homology to other proteins. The expression and possible functions of each of these receptor PTPases in the nervous system are described here and summarized in Table 1.

LAR (Leukocyte common Antigen Related protein)

LAR is a widely-expressed receptor PTPase, which was first identified because of its homology to CD45, and was later shown to have PTPase activity. Greater than 95% of the PTPase activity of LAR is associated with the first catalytic domain (Pot et al., 1991). Using a variety of artificial substrates, the second domain has been shown to have no intrinsic enzymatic activity (Streuli et al., 1990). Thus, it was suggested to have a regulatory function. However since the in vivo substrates of LAR are unknown, the possibility that the second domain has PTPase activity with suitable substrates or that such an activity needs to be activated by so far unknown process cannot be ruled out.

The extracellular domain of LAR contains three Ig-like repeats and eight FN III-like repeats (Fig. 1). The combination of Ig- and FN III-like repeats is also found in cell adhesion molecules like NCAM (Rutishauser, 1983) and TAG-1 (Furley et al., 1990), as well as in other receptor PTPases, PTPμ (Gebbink et al., 1991) and PTPκ (Jiang et al., 1993). NCAM, TAG-1, PTPμ and PTPκ all display homophilic binding properties (Brady-Kalnay et al., 1993; Gebbink et al., 1993; Sap et al., 1994). Thus, one might predict that LAR, too, can bind

homophilically; however, to date there are no reports of binding assays showing such a property.

Another interesting feature of LAR is the existence of proteolytically cleaved forms, which have been observed for both rat (Yu et al., 1992) and human LAR (Streuli et al., 1992). The extracellular 150 kDa portion of LAR containing the Ig- and FN III-like repeats can either be associated non-covalently with a transmembrane/intracellular portion on the cell surface or can be shed in cell culture. This cleavage occurs before the protein reaches the cell surface. The endopeptidase recognition site is five consecutive arginine residues located about 50 amino acids extracellular to the transmembrane domain. Proteolytic cleavage of LAR is not required for cell surface expression because mutations in the penta-arginine sequence prevent cleavage, but do not prevent surface expression (Streuli et al., 1992). Neither the mechanisms that regulate cleavage and shedding nor the effects of these phenomena on the phosphatase activity are known. Both NCAM and TAG-1 have membrane associated and soluble forms which have been suggested to play somewhat distinct roles in cell adhesion during neural development (Rutishauser and Jessell, 1988; Karagogeos et al., 1991). Thus, studies investigating the functions of soluble, as well as membrane-bound, LAR in the developing CNS will be essential.

The human LAR gene maps to chromosome 1p32, a region that is frequently deleted in neuroblastoma and pheochromocytoma (Jirik et al., 1992). Although the gene structure for LAR has not been established yet, extensive cDNA analysis demonstrates that the LAR gene has many alternative splice sites. In the CNS, different cassettes of amino acids can be inserted into both the extracellular and the intracellular domains (Zhang and Longo, in press). Furthermore, northern hybridization with LAR shows three major transcripts in

brain, with approximate sizes of 4.0, 6.0 and 8.0 kb. The ratio of expression of these three transcripts changes during cortical development (Chapter 1). In the embryonic cortex, the 4.0 kb transcript is the most abundant. During development, the 8.0 kb transcript is up-regulated, surpassing the smaller transcripts and reaching a peak in the adult cortex. The relative expression levels of the three transcripts is also different in different regions of the adult rat brain (Longo et al., 1993). The exact structure of the LAR gene that gives rise to these differentially-expressed transcripts awaits further studies.

While immunocytochemistry with LAR antibodies so far has shown no staining in the brain (Streuli et al., 1992), LAR mRNA can be detected abundantly in this tissue, both by northern and by in situ hybridization analyses (Longo et al., 1993; Sahin et al., 1995). In primary cultures, LAR is expressed by both neurons and glia; however, in the intact central and peripheral nervous systems, it is exclusively associated with cells with neuronal morphology (Longo et al., 1993). During development, LAR mRNA can be detected in the brain as early as E13 by PCR, and in situ hybridization shows a high level of LAR expression in proliferative zones at E17 (Sahin et al., 1995). Interestingly, LAR mRNA expression is up-regulated during NGF-induced neuronal differentiation of PC12 cells (Longo et al., 1993). Therefore, at least some of the diverse isoforms of LAR are expressed in patterns suggesting involvement in neuronal birth and in early stages of neuronal differentiation.

CPTP1 (Cortex-enriched Protein Tyrosine Phosphatase-1)

CPTP1 (Sahin and Hockfield, 1993) is a brain-enriched PTPase that was simultaneously identified from different tissues by six different groups, and so has also been named PTP NE-3 (Walton et al., 1993), PTP-P1 (Pan et al., 1993), RPTP-σ (Yan et al., 1993), LAR-PTP2 (Zhang et al., 1994) and CRYPα

(Stoker, 1994). Because one of the most important features of this gene is its brain enriched expression, we refer to it as CPTP1. The published full length CPTP1 cDNA clones all have 3 Ig-like and 5 FN III-like repeats (Fig. 1). More recently, a liver cDNA sequence that has three additional FN III-like repeats spliced into the extracellular domain of CPTP1 has been reported (Zhang et al., 1994). Thus, the liver isoform of CPTP1 has an extracellular region very similar in length and domain structure to LAR. Interestingly, on northern blots of rat neocortex RNA, CPTP1 hybridizes to two bands of 6.5 and 8.0 kb (Sahin and Hockfield, 1993), suggesting that the difference between these bands might be attributable to the number of FN III-like repeats. In fact, there is preliminary evidence that two forms of CPTP1 differing in the number of their FN III-like repeats are expressed in the brain (Zhang et al., 1994). Furthermore, in PC12 cells, there is a 4.8 kb transcript that is generated from the CPTP1 gene by alternative splicing (Pan et al., 1993). This isoform is identical to the full length sequence, except that it lacks the entire second PTPase domain. In the chick, CPTP1 homolog appears to give rise to at least four alternatively spliced transcripts (Stoker, 1994). Genomic southern analysis strongly suggests that there is only one CPTP1 gene (Walton et al., 1993; Sahin and Hockfield, 1993; Stoker, 1994), which is localized to mouse chromosome 17 (Yan et al., 1993) and human chromosome 19 (preliminary results, M.S., Susan Slaugenhaupt, Susan Hockfield). Therefore, multiple transcripts must reflect alternative splice and/or polyadenylation sites. Confirmation of this awaits more extensive genomic and cDNA analyses.

While most of the other receptor PTPases are widely expressed, CPTP1 is markedly enriched in the adult brain compared to non-neural tissues. In fact, one of its transcripts (6.5 kb mRNA) appears to be exclusively expressed in neural tissues (Sahin and Hockfield, 1993; Stoker, 1994; Sahin et al., 1995).

Moreover, while the 8.0 kb mRNA is detected in other tissues, it is expressed at a far greater level in the brain. In the adult CNS, CPTP1 is expressed in the hippocampus, neocortex, entorhinal cortex, olfactory bulb, cerebellum and spinal gray matter. During development, CPTP1 is detected throughout the developing CNS, including the retina, and in the dorsal root and trigeminal ganglia (Sahin and Hockfield, 1993; Yan et al., 1993). In all areas that have been studied, CPTP1 mRNA is associated with neurons, and not with glia (Walton et al., 1993; Sahin and Hockfield, 1993). In the olfactory epithelium, CPTP1 is expressed by both neuronal precursors and mature sensory neurons (Walton et al., 1993). Similarly, within the embryonic forebrain, CPTP1 is found in both the proliferative zone and the cortical plate, indicating expression by neuronal precursors and by early post-mitotic neurons, respectively (Sahin and Hockfield, 1993). The detection of CPTP1 as early as E12-13 in the developing CNS is consistent with expression by progenitors and newly born neurons (Yan et al., 1993; Sahin et al., 1995). The possibility that CPTP1 plays a role in early neuronal differentiation is strengthened by the observation that CPTP1 mRNA is up-regulated during NGF-induced neuronal differentiation of PC12 cells (Pan et al., 1993).

Antisera raised against the extracellular domain of CPTP1 recognize two bands on western blots, at 100 and 200 kDa. The higher molecular band is thought to represent the full length protein product while the 100 kDa band may represent the proteolytically cleaved extracellular domain (Yan et al., 1993). However, unlike LAR, a cleavage site in the extracellular domain of CPTP1 has not been identified. Using a peptide located at the N-terminus of CPTP1, we have generated a rabbit antiserum that recognizes a 100 kd band on western blots (data not shown). Immunoblot staining can be blocked by the appropriate peptide, but not by an unrelated peptide. Although it is thought to correspond to

the cleaved extracellular domain of CPTP1, the 100 kDa band is detected in the particulate, not in the soluble fraction of brain homogenates. This observation is consistent with the membrane association of this polypeptide in the lung (Rotin et al., 1994). Furthermore, the expression of this peptide follows closely the temporal and spatial distribution of CPTP1 mRNA, i.e. immunostaining is highly enriched in the brain and is down-regulated during development. While this antiserum appears to recognize the right protein product on western blots, it does not work for immunocytochemical localization of the CPTP1 protein. Interestingly, immunocytochemistry with antibodies raised to the chick homolog of CPTP1 indicates that this protein is localized to growth cones of a subset of embryonic axons (Stoker et al., 1994, Soc. Neurosci., abstract), providing further evidence that CPTP1 may play a role in neurite outgrowth and axon guidance.

PTPδ

While human PTPδ (HPTPδ) was cloned in 1990 by Krueger et al., an analysis of tissue specific expression was not performed until the murine homolog (MPTPδ) was isolated (Mizuno et al., 1993). While the full length amino acid sequence of MPTPδ shows 69% identity to LAR, its expression is strikingly different from LAR. LAR is found predominantly in T-cells and in cells of epithelial origin. In contrast, MPTPδ is restricted to brain, kidney, heart and B-cells. In the lung, where LAR is expressed at very high levels, MPTPδ is not detected (Sahin et al., 1995; Mizuno et al., 1993). Furthermore, MPTPδ maps close to locus b on mouse chromosome 4, which does not correspond to the chromosomal location of the human LAR gene. Therefore, despite high sequence homology to LAR, PTPδ appears to be a distinct gene.

Sequencing analysis of various cDNAs indicate that there are at least

three transcripts of MPTPδ (Mizuno et al., 1993). All three transcripts share the same transmembrane and phosphatase domains, but differ in their extracellular domains (Fig. 1): type A contains one Ig-like and four FN III-like domains; type B contains one Ig-like and eight FN III-like domains; type C contains three Ig-like and eight FN III-like domains. So far, the three transcripts do not appear to be differentially expressed. Furthermore, an antiserum raised to a region shared among the three isoforms recognizes only one protein (about 210 kDa) on western blots (Mizuno et al., 1993). Therefore, it is not clear whether all three isoforms are translated into mature protein products. Further studies with isoform specific cDNA probes and antibodies will be required to answer this question.

In situ hybridization analysis with MPTPδ demonstrates that it is expressed in the adult hippocampus, thalamus and piriform cortex. However, to date there is no information about the expression of MPTPδ in the developing CNS. It will be very interesting to compare the expression patterns of the various LAR, CPTP1 and MPTPδ isoforms during CNS development.

PTPζ

PTPζ (also known as RPTPβ) has two phosphatase domains homologous to LAR, CPTP1 and PTPδ, but has a divergent extracellular domain (Krueger and Saito, 1992; Levy et al., 1993). Instead of several Ig- and FN III-like repeats, the most N-terminal extracellular portion of PTPζ shows homology to the enzyme carbonic anhydrase (CA). This region is followed by one FN III-like repeat and a large cysteine-free domain that has no homology to other proteins (Fig. 1). Within the CA domain, the residues that are critical for zinc binding and catalytic activity of CA are missing from PTPζ. However, this domain contains a hydrophobic pocket that has been suggested to be a binding

site for small molecules, including short peptides (Krueger and Saito, 1992).

Northern and cDNA analyses (Barnea et al., 1993; Barnea et al., 1994b) demonstrate that there are three transcripts of PTPζ (9.5, 8.5 and 6.4 kb), probably generated by alternative splicing from a single gene located on human chromosome 7q31-33 (Levy et al., 1993). The 9.5 kb transcript encodes the full length transmembrane form (Fig. 1). The 6.4 kb transcript also encodes a transmembrane isoform, but it lacks an 860 amino acid stretch in the cysteine-free extracellular region. The 8.5 kb transcript encodes a secreted form that corresponds to the full length extracellular domain of PTPζ without the transmembrane and intracellular domains. This isoform of PTPζ shows high homology to a previously identified rat proteoglycan, 3F8 PG (Barnea et al., 1994b). Consistent with this homology, fibroblast cell lines transfected with the human 9.5 kb isoform express a chondroitin sulfate proteoglycan (Barnea et al., 1994a). Thus, the 3F8 PG is likely to be the rat homolog of the secreted form of human PTPζ. In aggregation assays, the 3F8 PG binds tenascin (Barnea et al., 1994a; Grumet et al., 1994) and inhibits the homophilic binding of NCAM and NgCAM (Grumet et al., 1993), leading to the suggestion that at least some isoforms of PTPζ may also bind these neural cell adhesion and extracellular matrix molecules. So far, aggregation experiments have not been reported with any of the isoforms of PTPζ. Somewhat paradoxically, an antisera raised to the C-terminal domain shared between the two transmembrane forms of PTPζ immunoprecipitates only a single high molecular weight species. Thus, the biochemical identification of the protein products corresponding to the alternatively spliced transcripts awaits further investigation.

Similar to CPTP1, PTPζ expression is restricted to the nervous system (Barnea et al., 1993), where it can be detected as early as E12 (Canoll et al., 1993). The identity of the cells producing PTPζ has been controversial because

of conflicting reports of mRNA expression: PCR shows PTPζ mRNA highly enriched in glioma, but not in neuroblastoma cell lines (Krueger and Saito, 1992); northern hybridization shows high levels in neuroblastoma, but not in glioblastoma cell lines (Barnea et al., 1993). Immunocytochemistry with antisera raised to the transmembrane forms of PTPζ indicates that it is highly expressed on radial processes of radial glia during development (Canoll et al., 1993). PTPζ immunoreactivity is also associated with the glia of the embryonic optic stalk and of adult olfactory bulb axons, as well as with the Bergmann glia in the adult cerebellum. Preliminary evidence indicates that different isoforms of PTPζ are expressed by different types of glial cells (Canoll et al., 1994, Soc. Neurosci., abstract). Thus, PTPζ appears to be expressed by various types of glial cells and could play a role in neuron-glial interactions.

RPTPγ

Like PTPζ, RPTPγ has a large extracellular region containing a CA domain, a FN III-like domain and a cysteine-free domain (Barnea et al., 1993) (Fig. 1). The homology between PTPζ and RPTPγ also extends into the cytoplasmic domains. Thus, these two PTPases define a subfamily of receptor PTPases, but extracellular ligands for RPTPγ have not yet been identified. In contrast to PTPζ, RPTPγ is not restricted to the CNS. In the rat, RPTPγ is expressed at about equal levels in the brain, heart, lung, kidney and ovary (Barnea et al., 1993; Sahin et al., 1995). The human RPTPγ gene (HPTPγ) maps to a region on chromosome 3 that is frequently deleted in lung and renal cell carcinomas (Laforgia et al., 1991). These two lines of evidence have led to the suggestion that RPTPγ may play a tumor-suppressive role in these organs.

The function of RPTPγ in the developing brain is far from clear. Northern blot analysis demonstrates two major high molecular weight transcripts around

7.0 and 9.0 kb, and two minor transcripts with molecular weights around 4.3 and 3.3 kb (Chapter 1). The two major transcripts display parallel temporal regulation patterns, both reaching their highest levels in the second postnatal week and then gradually declining to adult levels. Especially intriguing is the brain-specific 3.3 kb mRNA, which is expressed at moderate levels at E16 and is then markedly down-regulated postnatally. The 4.3 kb mRNA does not appear to be temporally regulated. The cDNAs corresponding to each of these transcripts have not been identified. Perhaps of greatest interest is that the expression pattern of RPTPγ in the CNS is unique among the PTPases. At E17, RPTPγ is expressed at higher levels in the midbrain than in the cortex, while at P4 it is expressed at a higher level in the cortex than the midbrain (Chapter 1). During the embryonic period post-mitotic neurons express higher levels of RPTPγ than do proliferating cells. Also unlike the other PTPases, in the early postnatal rat, RPTPγ expression in the neocortex is restricted to the superficial layers (Sahin et al., 1995). Neurons in these layers are still migrating to their final position. Thus, RPTPγ may be involved in neuronal migration. However, the fact that RPTPγ mRNA levels increase over the course of cortical development suggests that its role may be more closely related to constitutive neuronal functions, perhaps serving a tumor suppressor-like function.

LRP (Leukocyte common antigen Related Phosphatase)

Unlike the rest of the receptor PTPases discussed in this chapter, LRP (also known as PTPα) has an extracellular domain that does not contain cell adhesion molecule-like motifs. Instead, its 123 amino acid extracellular domain has multiple glycosylation sites similar to the N-terminal region of CD45, suggesting that LRP may present carbohydrates to lectins (Matthews et al., 1990). Like CD45, LRP could have different interactions and functions

depending on its glycosylation pattern. This hypothesis will be testable when the carbohydrate groups present on LRP are identified, as recently reported for CD45 (Sato et al., 1993). It has already been shown that LRP cDNA can give rise to at least two protein species, which differ in their glycosylation pattern (Daum et al., 1994). Pulse chase experiments indicate that a smaller protein precursor gives rise to a larger form, although it is not known if the smaller precursor is expressed on cell surfaces.

CD45 and LRP are the only receptor PTPases for which the genomic structures have been completely elucidated. The gene structures of CD45 and LRP are strikingly similar in size and organization (Wong et al., 1993). The LRP gene maps to mouse chromosome 2 and contains 22 exons spanning over 75 kb. The first and second phosphatase domains are made up of eight and seven exons, respectively. The active site (HCSAG) is found at the junction of two exons. Inclusion of exon 8, which encodes an 108 amino acid insert, into the first catalytic domain leads to diminished PTPase activity (Matthews et al., 1990). A cDNA clone containing a 9 amino acid insert in the extracellular segment has been identified (Kaplan et al., 1990) from a human brainstem library. mRNAs with this insert are found in a variety of tissues, but at lower abundance than mRNAs without this insert (Daum et al., 1994). Because the protein products of the two cDNA are identical in size, PTPase activity and glycosylation pattern, the significance of the alternative splicing is not known. One possibility is that the two isoforms will show subtle differences in extracellular interactions, which may become clear once the physiological ligands are identified.

LRP is widely expressed in various tissues (Sahin et al., 1995), and the LRP promoter is similar to promoters found in widely expressed genes involved in cellular metabolism and growth (Wong et al., 1993). Among adult tissues, the

brain expresses the highest level of LRP. Lung and ovary have intermediate levels while heart and kidney have low levels of LRP mRNA. In the developing cerebral cortex, LRP mRNA is expressed at relatively constant levels from embryos to adults (Chapter 1). Experiments with cell lines in vitro suggest a role for LRP in early stages of neural differentiation. LRP expression is up-regulated during neuronal differentiation of embryonal carcinoma and neuroblastoma cell lines (Den Hertog et al., 1993). Maximal expression of LRP is reached immediately before the neuronal phenotype is displayed. In addition, overexpression of LRP in P19 embryonal carcinoma cell line produces a higher incidence of neuronal differentiation and the acquisition of more mature neuronal properties such as electrical excitability (Den Hertog et al., 1993). Enhancement of src kinase activity in LRP-transfected cells is thought to underlie this effect. LRP is known to activate src in other cell lines like fibroblasts, possibly by dephosphorylating its C-terminal autoinhibitory phosphorylation site (Zheng et al., 1992). Expressed at about equal levels throughout development, LRP may have distinct roles in the developing and mature CNS.

Other receptor PTPases in the brain:

The list of receptor PTPases expressed in the developing mammalian brain has grown rapidly in the last few years. However, there is reason to believe that the full repertoire of receptor PTPases expressed in the developing CNS has not yet been revealed. For example, DPTP10D is expressed in the Drosophila CNS, but little is known about the expression of its mammalian homolog PTPβ. CD45 has been reported to be expressed by both resting and activated microglia in human brain (Masliah et al., 1991). PTPε, a receptor PTPase with similarity to LRP, has been detected in the olfactory epithelium by PCR (Walton

et al., 1993), but its expression in the rest of the nervous system has not been examined. In addition to these previously cloned PTPases, there may also be as yet unidentified PTPases that do not contain the conserved sequences upon which PCR primers have been designed in the studies carried out so far. The identification of such PTPases awaits biochemical characterization and/or functional screening methods like those used to identify the first members of the PTPase family.

NON-RECEPTOR PTPases IN THE CNS:

Non-receptor PTPases characterized to date can be divided into five subfamilies depending on their sequence outside of the PTPase domains: (1) placental PTP1B (Chernoff et al., 1990; Brown-Shimer et al., 1990) and its homologs, T-cell PTPase (Cool et al., 1989) and rat PTP-1 (Guan et al., 1990); (2) striatum enriched phosphatase, STEP (Lombroso et al., 1991); (3) the SH2 domain containing PTPases, SHP (Shen et al., 1991; Yi et al., 1992; Plutzksy et al., 1992; Matthews et al., 1992) and Syp (Hiraga et al., 1992; Freeman et al., 1992; Adachi et al., 1992; Feng et al., 1993; Vogel et al., 1993; Ahmad et al., 1993) (4) PEST sequence containing PTPases, PEP (Matthews et al., 1992) and P19-PTP (Den Hertog et al., 1992; Takekawa et al., 1992; Yang et al., 1993; Sahin and Hockfield, 1993); (4) PTPases with homology to cytoskeleton associated proteins, PTPH1 (Yang and Tonks, 1991), MEG (Gu et al., 1991), PTP36 (Sawada et al., 1994) and hPTP1E (Banville et al., 1994). Of these genes, PTP-1, STEP, Syp, SHP, P19-PTP and PTPH1 are known to be expressed in the brain and are discussed below:

PTP-1

Originally cloned from a hypothalamic cDNA library, PTP-1 is the rat

homolog of human PTP-1B, which was the first biochemically purified PTPase (Charbonneau et al., 1989). PTP-1 sequence contains a catalytic domain at its N-terminus and a hydrophobic stretch of amino acids at its C-terminus (Fig. 2). Rat PTP-1 and human PTP1B sequences only differ significantly in their C-terminal ends. The C-terminus of PTP1B have been shown to be necessary and sufficient for its targeting to the cytoplasmic face of the ER (Frangioni et al., 1992). Alternative splicing of PTP1B at its C-terminus has been reported although its effect on targeting to the ER is not clear (Shifrin and Neel, 1993). Thus, the subcellular localization of PTP-1 awaits further studies.

PTP-1 gene is located on human chromosome 20 and gives rise to a 3.5 kb mRNA (Brown-Shimer et al., 1990). PTP-1 mRNA is broadly expressed in a number of tissues, at particularly high levels in the lung (Chernoff et al., 1990; Rotin et al., 1994). Within the CNS, PTP-1 mRNA is detected at E16 throughout the brain (Chapter 1) while in the adult it is restricted to the hippocampus (Guan et al., 1990).

STEP

STEP was originally identified by a subtractive hybridization approach looking for striatum specific cDNAs (Lombroso et al., 1991). Unlike most other non-receptor PTPases, the catalytic domain of STEP is located at the C-terminus (Fig. 2). The non-catalytic sequences of STEP are unrelated to sequences in the databases. The STEP gene has at least two alternatively spliced forms. The 4.4 kb mRNA is present in many brain areas while 3.0 kb band is particularly enriched in the striatum (Lombroso et al., 1991). Alternatively spliced STEP transcripts have been recently identified (Lombroso et al., 1994, Soc. Neurosci., abstract). These mRNAs give rise to four polypeptides (33, 37, 42 and 65 kDa) which vary in their subcellular localization

and regional expression patterns (Boulanger et al., in press). The three smaller polypeptides are enriched in the soluble fraction, while the 65 kDa polypeptide is in the particulate fraction of brain homogenates. In the striatum, STEP is expressed by medium spiny neurons that receive dopaminergic input and co-localizes with DARPP-32. STEP protein is found in the cell bodies of these neurons as well as in their axon terminals in target areas such as substantia nigra. STEP immunoreactive cell bodies are also found in the nucleus accumbens, olfactory tubercle, lateral septal nucleus, and cortex (Lombroso et al., 1993). STEP is not detected in the embryo and is expressed in the striatum only after birth (Raghunathan et al., 1994, Soc. Neurosci., abstract). The relatively late developmental expression of STEP and its co-localization with DARPP-32 in dopaminoceptive neurons have lead to the suggestion that it plays a role in neurotransmitter signaling (Naegele and Lombroso, in press).

SHP

SHP has been identified by a number of groups from different tissues and given different names: PTP1C from breast carcinoma (Shen et al., 1991); HCP from myeloid cells (Yi et al., 1992); SH-PTP1 from megakaryocytes (Plutzksy et al., 1992); SHP from pre-B-cells (Matthews et al., 1992). Its sequence indicates an N-terminus containing two SH2 domains, followed by a catalytic domain and a short C-terminal non-catalytic segment (Fig. 2). The 2.6 kb SHP transcript is expressed predominantly in hematopoietic cells, particularly in the thymus and bone marrow (Yi et al., 1992). However, it is also found in other tissues such as the lung and the brain (Chapter 1). Within the brain, SHP is expressed by cells of hematopoietic origin, such as microglia and macrophages.

SHP gene is located on human chromosome 12 and mouse

chromosome 6 (Yi et al., 1992). The human gene maps to a region associated with acute lymphocytic leukemias. In mice, an immunodeficient phenotype, *motheaten (me)*, results from a loss-of function mutation in the SHP gene (Shultz et al., 1993; Kozlowski et al., 1993). *Me* mice do not produce any SHP protein and demonstrate severe defects in hematopoiesis and immune function, such as hyperproliferation of macrophages. Thus, it has been suggested that SHP may be an important negative regulator of hematopoietic cell proliferation possibly by modulating cytokine initiated tyrosine phosphorylation (Kozlowski et al., 1993; Sun and Tonks, 1994). The defects in *me* mice also indicate that other non-receptor PTPases do not compensate for the loss of SHP activity and support the suggestion that different PTPases have specific substrates and functions within a cell. The effects of this mutation on SHP expression in the brain and on microglia phenotype are not known.

Given its pivotal role in hematopoiesis, structure-function analysis of SHP will be an intensely explored topic. SH2 domains are present in many molecules involved in signaling pathways and are known to bind specific phosphotyrosine residues (Songyang et al., 1993). Several studies have demonstrated that SH2 domains of SHP can interact with certain phosphotyrosine residues (Shen et al., 1991; Matthews et al., 1992; Vogel et al., 1993). Multiple isoforms of SHP can be generated by alternative splicing at the 5' terminus although the functional differences between the various isoforms are not known (Feng and Pawson, 1994).

Syp

Unlike SHP, which is expressed predominantly in hematopoietic cells, Syp (also known as PTPase L1, SH-PTP2, SH-PTP3, PTP1D, PTP2C) is expressed ubiquitously. Full length Syp cDNAs has been isolated from liver

(Hiraga et al., 1992), brain (Freeman et al., 1992), T-cell line (Adachi et al., 1992), breast carcinoma cell line (Feng et al., 1993; Vogel et al., 1993), umbilical cord (Ahmad et al., 1993). Syp RNA is detected in embryonic heart and brain as well as adult brain, heart, kidney, liver, lung and spleen (Feng et al., 1993). On northern blots, a 6 kb transcript is detected. Open reading frame is only 1.8 kb while the rest of the transcript consists of a long 3' untranslated region.

The protein structure of Syp is similar to that of SHP: two SH2 domains at the N-terminal followed by a catalytic domain (Fig. 2). Thus, SHP, Syp and Drosophila PTPase corkscrew (csw) form a subfamily of non-receptor PTPases (Feng and Pawson, 1994). Interestingly, the C-terminal segment of Syp is more similar to corkscrew than to SHP, and like csw, it is expressed broadly. Thus, csw and Syp are probably species homologs. csw is known to act with raf in the signal transduction pathway initiated by a receptor PTK, torso (Perkins et al., 1992). Syp can play a similar role by acting downstream of receptors for many growth factors including insulin, IGF-1 and EGF (Xiao et al., 1994). Interfering with Syp function in these systems results in a dramatic decrease in DNA synthesis and mitogenic signaling through growth factor receptors. The sequence of events in this pathway is thought to be as follows: Ligand binding causes autophosphorylation of the receptor PTKs. Syp binds autophosphorylated receptor PTKs, like EGF and PDGF receptors, Her2-neu and kit-SCF and gets tyrosine phosphorylated (Feng et al., 1993; Vogel et al., 1993). Then, phosphorylated Syp acts as an adapter molecule and binds Grb2 (Li et al., 1994; Bennett et al., 1994). GRB2 binding via Syp links ligand binding to the growth factor receptor to ras activation.

In addition to its role as as an adapter molecule, Syp plays a catalytic role in signaling as well. T he PTPase activity of Syp can be regulated by its

interaction with receptor PTKs such that tyrosine phosphorylation of Syp enhances its catalytic activity (Vogel et al., 1993; Uchida et al., 1994). Interestingly, Syp does not seem to dephosphorylate the PTKs that it associates with (Vogel et al., 1993). An RNA splice variant which contains four amino acid insert in the catalytic domain has been identified (Mei et al., 1994). This variant has lower catalytic activity indicating that alternative splicing may be a regulatory mechanism for PTPase activity. While its substrates have not been identified, Syp has been shown to preferentially dephosphorylate tubulin (Hiraga et al., 1992). With a putative role in FGF receptor signaling pathway (Sun and Tonks, 1994) and ability to dephosphorylate tubulin, Syp may also be involved in neurite outgrowth (see below).

P19-PTP

P19-PTP cDNA sequence indicates an N-terminal segment containing the catalytic domain and a C-terminal segment rich in proline, serine, threonine and acidic residues such as glutamate and aspartate i.e. a PEST sequence (Fig. 2). PEST sequences are also found in another PTPase, PEP, which is expressed primarily in hematopoietic cells (Matthews et al., 1992). Many nuclear proteins such as c-myc and c-fos contain PEST sequences, raising the possibility that P19-PTP and PEP may be nuclear. Recent experiments show that PEP is indeeed localized to the nucleus (Flores et al., 1994). Nuclear targeting signal is in the last 18 amino acids at the C-terminus of PEP. P19- PTP also contains a similar stretch of peptide sequence at the extreme C-terminus, suggesting that it also localizes to the nucleus. PEST sequences are characteristic of proteins with short half-lives; however, pulse-chase experiments show that PEP has a half-life greater than five hours (Flores et al., 1994). Currently, both the subcellular localization or the half-life of P19-PTP

remain to be determined experimentally.

Like many PTPases, P19-PTP is a phosphoprotein. Phosphorylation by PKA or PKC on a serine residue decreases the phosphatase activity of P19-PTP (Garton and Tonks, 1994). P19-PTP expression is also highly regulated at the mRNA level. In P19 embryonal carcinoma cell system, P19-PTP mRNA is only expressed by cell aggregates, not by monolayers (Den Hertog et al., 1992). In skeletal muscle cell lines, insulin causes an increase of P19-PTP mRNA (Yang et al., 1993). At the same time, P19-PTP mRNA is broadly distributed in a variety of tissues. Within the brain, its expression is highest during embryonic development suggesting a role during neuronal proliferation and early differentiation (Sahin et al., 1995).

PTPH1

PTPH1 sequence contains a N-terminal domain with homology to cytoskeleton-associated proteins such as band 4.1, ezrin and talin while its C-terminus encodes a phosphatase domain (Fig. 2). Because of its homology to cytoskeleton-associated proteins, it has been suggested that PTPH1 may "act at the interface between the plasma membrane and the cytoskeleton, for instance at focal adhesion plaques (Yang and Tonks, 1991)." This is a tempting idea because PTKs are heavily concentrated at such plaques. However, to date there is no report of its subcellular localization.

On northern blots probed with PTPH1, a single 4.3 kb band is detected in HeLa cells (Yang and Tonks, 1991) and in the thalamus (Chapter 3). The gene is located on human chromosome 9, close to the locus of familial dysautonomia (Blumenfeld et al., 1993). Given its expression in dorsal root ganglia during early development, PTPH1 is a good candidate gene for this disorder (Axelrod, 1984; Pearson, 1984). However, currently no mutations have been detected in

the open reading frame of PTPH1 in patients with familial dysautonomia (Susan Slaugenhaupt, personal communication). Whether there are mutations in the non-coding regions of PTPH1 remain to be determined.

FUNCTIONAL ROLES OF PTPases

While new members of the PTPases family are being cloned and sequenced at a staggering pace, the physiological roles played by these newly identified enzymes remain largely unknown. One obvious role is that PTPases can act as negative regulators and directly oppose the actions of PTKs in cells, as in the case of PTP1B blocking oncogenic transformation by PTKs (Brown-Shimer et al., 1992). This counter- regulatory role requires a dynamic balance between the activities of PTKs and PTPases, altering the phosphorylation state of cellular proteins. In contrast, PTPases can also take part in the same pathway and work in concert with PTKs. For instance, CD45 and LRP dephosphorylate a regulatory tyrosine on src family kinases to activate these PTKs (Koretzky, 1993; Zheng et al., 1992). Similarly, receptor PTKs phosphorylate non-receptor PTPases and modulate their activity (Vogel et al., 1993; Maegawa et al., 1994). Therefore, PTKs and PTPases may regulate each other by phosphorylation and thus act in a coordinated manner in cellular signaling. This is best demonstrated in the case of CD45 and T-cell receptor signaling (Chan et al., 1994).

CD45

The receptor PTPase that has been most extensively studied is CD45 (reviewed in Koretzky, 1993; Trowbridge and Thomas, 1994). CD45, also known as Leukocyte Common Antigen (LCA), is expressed by all nucleated cells of the hematopoietic lineage. The CD45 gene gives rise to diverse isoforms by alternative splicing of exons encoding the 5' portion of the

extracellular domain as well as by a variety of differential glycosylation patterns (Thomas, 1989). CD45 mRNA splicing is regulated in both a cell type-specific and developmental pattern and can be altered by T-cell activation (Janeway, 1992; Trowbridge and Thomas, 1994).

Recent studies have sought to identify CD45's ligands, its intracellular associations and the mechanism of regulation of its catalytic activity. One CD45 isoform expressed on helper T-cells, CD45RO, binds CD22β on B cells (Stamenkovic et al., 1991). CD22β is a member of the Ig superfamily and is a lectin that interacts with α-2,6-linked sialic acids on many proteins including CD45 (Sgroi et al., 1993; Powell et al., 1993). Structural studies of CD45 have confirmed that at least some of its isoforms contain α-2,6-linked sialic acid residues (Sato et al., 1993). Thus, the binding of CD45 isoforms to CD22β or other ligands will most likely depend on their glycosylation pattern. Meanwhile, there is no evidence that CD22β modulates the activity of CD45 (see Trowbridge and Thomas, 1994, for a detailed discussion).

Intracellularly, CD45 can activate the cytoplasmic PTKs, lck and fyn, by dephosphorylating tyrosine residues in their regulatory domains (Woodford-Thomas and Thomas, 1993). lck is associated with CD4 and CD8 while fyn is associated with the T-cell receptor (TCR) (for review see (Rudd et al., 1993; Chan et al., 1994)). The first biochemical effect of TCR stimulation is an induction of PTK activity (Saouaf et al., 1994). Since the TCR has no intrinsic PTK activity, it is thought that activation of non-receptor PTKs by CD45 mediates this step. Subsequent tyrosine phosphorylation of cytoplasmic proteins, in particular of phospholipase C, results in increased phosphotidyl inositol turnover, protein kinase C activation, a rise in intracellular calcium levels and cytoskeletal reorganization. Cell culture experiments with $CD45^-$ mutants and whole animal gene knock out experiments have shown that CD45 is essential

for T-cell activity and development (Koretzky, 1993; Desai et al., 1993; Kishikara et al., 1993). Aside from the TCR complex and non-receptor PTKs, CD45 seems to be associated with other cytoplasmic proteins. Some of these, like CD45-AP, are novel putative adapter proteins (Takeda et al., 1994) while others, like fodrin and spectrin, are well-known cytoskeletal elements (Lokeshwar and Bouguignon, 1992).

While extracellular and cytoplasmic interactions of CD45 are beginning to be understood, little is known about how the PTPase activity of CD45 is regulated. Intracellularly, the binding of fodrin to CD45 stimulates its PTPase activity (Lokeshwar and Bouguignon, 1992); however, the role of the extracellular domain is far from clear. By expressing just the intracellular portion of CD45 in $CD45^-$ cell lines, two groups have shown that the transmembrane and extracellular portions of CD45 are not required in the activation of T-cells by antibodies to the TCR (Hovis et al., 1993; Volerevic et al., 1993). Studies with chimeric proteins composed of the EGF receptor extracellular domain and the CD45 intracellular domain suggest that the CD45 catalytic domain is constitutively active (Desai et al., 1993; Desai et al., 1994). Furthermore, ligands for this chimeric protein, such as EGF, inhibit signaling through the TCR, possibly through dimerization of the chimeric proteins. This inhibition could be mediated by a decrease in PTPase activity of CD45 or by a blockade of its ability to interact with its substrates. So far there is no direct evidence for regulation of CD45 catalytic activity in response to EGF in this system. Interestingly, recent studies with fibroblast cell lines indicate that extracellular binding can modulate phosphatase activity of other receptor PTPases. In that case, PTPases are activated when cell-substrate adhesion is disrupted (Maher, 1993). This suggests that down-regulation of catalytic activity by ligand binding may be a property shared by CD45 and other receptor PTPases. Unlike many

other receptors including receptor PTKs, for which ligand binding activates signal transduction, signal transduction through receptor PTPases may be negatively regulated by ligand binding. Such a regulatory mechanism is quite attractive for PTPases because their intrinsic catalytic activities are strikingly high (Fischer et al., 1991).

Neurite outgrowth

Guided and stimulated by the progress in the immune system, the study of cellular interactions during neural development is an expanding area of research. Recently developed in vitro assay systems have facilitated the analysis of processes such as neuronal migration and neurite outgrowth (Rakic et al., 1994; Hynes and Lander, 1992). In both cases, it is becoming clear that complex and sometimes redundant molecular interactions underlie cellular events ranging from surface recognition/adhesion to transmembrane signalling to cytoskeletal modifications. We will briefly review the growing evidence suggesting that tyrosine phosphorylation/dephosphorylation plays a role in signal transduction pathways that regulate neurite outgrowth in both non-vertebrate and vertebrate systems.

In Drosophila, null mutations of either the cell adhesion molecule, fasciclin I (fas I), or a cytoplasmic tyrosine kinase, abl, have little observable effect on the nervous system. However, embryos double mutant for fasciclin I and abl show a pronounced disorganization of the nervous system, presumably due to a developmental misrouting of growth cones (Elkins et al., 1990). This suggests that fas I and abl belong to two redundant and interacting pathways that converge on pathfinding in the growth cones. As already mentioned, other experiments performed in Drosophila have identified three transmembrane PTPases that are expressed on subsets of growing axons (Yang et al., 1991;

Tian et al., 1991; Hariharan et al., 1991) implying that tyrosine dephosphorylation is also involved in neurite outgrowth. Given the similar patterns of expression of abl and PTPases, it has been suggested that the PTPases may regulate the kinase activity of abl by modifying its phosphorylation state (Yang et al., 1991; Tian et al., 1991). So far, there is no direct evidence for any association between abl and the three Drosophila receptor PTPases.

Study of Aplysia neurons in culture indicates that tyrosine phosphorylated proteins are concentrated at the tips of growth cone filopodia and most likely associated with actin filaments (Wu and Goldberg, 1993). On growth promoting substrates, the phosphotyrosine staining of the filopodia tips is markedly reduced compared to less hospitable substrates such as polylysine. Conversely, inhibiting PTK activity with genistein decreases tyrosine phosphorylation and causes rapid elongation of filopodia. Removal of genistein is followed by shortening of the filopodia suggesting that actin filament dynamics are linked to the phosphorylation state of proteins in the growth cone. In this system, dephosphorylation increases neurite elongation while phosphorylation inhibits it. Some actin associated proteins, such as vinculin, can be phosphorylated in growth cones (Igarashi et al., 1990), providing a possible mechanism mediating cytoskeletal organization in response to extracellular substrates.

Several lines of evidence indicate that tyrosine phosphorylation can regulate neurite growth in similar ways in vertebrate neurons. First, cytoplasmic PTKs (src, fyn and yes) are enriched in growth cones (Maness et al., 1988; Bixby and Jhabvala, 1993). These PTKs are expressed at much higher levels during axonal growth than in the adult. Furthermore, expression of a constitutively active *v-src* gene in PC12 cells results in neurite outgrowth in the

absence of normally required NGF (Cox and Maness, 1993). Second, similar to Aplysia neurons, inhibition of PTKs by genistein facilitates substrate-induced neurite outgrowth in chick ciliary ganglion neuronal cultures (Bixby and Jhabvala, 1992), in NGF-primed PC12 cells (Miller et al., 1993) and in embryonic rat dorsal root ganglia cultures (Fryer et al., 1993, Soc. Neurosci., abstract). In goldfish retinal ganglion cell explants, genistein blocks antibody-induced axonal retraction (Finnegan et al., 1993). Thus, PTKs appear to be involved in both positive and negative regulation of neurite extension.

In order to assess the contribution of different PTKs to neurite outgrowth, Maness and her colleagues have analyzed neurite outgrowth from cells lacking a particular PTK. Comparisons between cerebellar granule cells from *src, yes* and *fyn* knock-out mice demonstrated that cells lacking src have impaired neurite outgrowth on the cell adhesion molecule L1 (Ignelzi et al., 1994) while those lacking fyn have a similar response on NCAM (Beggs et al., 1994). Growth cone morphology and filopodial length were not altered in this assay, implying a lack of effect on actin dynamics. In addition, neurite outgrowth on laminin was not perturbed, suggesting a specific interaction between src and L1 as well as fyn and NCAM. Interestingly, the same group previously demonstrated that binding of L1 or NCAM to growth cone membranes reduces tyrosine phosphorylation of tubulin (Atashi et al., 1992). Therefore, cell adhesion molecules like L1 and NCAM appear to act through src family kinases and at the same time reduce the phosphorylation of tubulin. These apparently contradictory results can be resolved if L1 binding activates a PTPase which, in turn, dephosphorylates a number of intracellular substrates, including tubulin and src.

As a result of their studies of neurite outgrowth on fibroblasts expressing neural cell adhesion molecules, Doherty and his colleagues have proposed

that FGF receptor may mediate the intracellular signaling from cell adhesion molecules like L1 and NCAM (Doherty and Walsh, 1994). Since Syp has been shown to interact with FGF receptor and highly expressed in the developing nervous system, Syp may also be involved in neurite outgrowth. Although the exact molecular mechanisms are not understood, these data lend support to the idea that tyrosine phosphorylation and dephosphorylation, regulated by extracellular signals, are involved in neurite outgrowth.

Biochemical cascades involved in neurite outgrowth

The receptor PTPases identified so far are expressed at high levels in the immune and developing nervous system. In both of these cases, cellular interactions are crucial to the function and development of the system. Using the parallels between the two systems, we can speculate about the involvement of PTPases in the biochemical cascades underlying T-cell activation and neurite outgrowth.

Like T-cell activation described above, neurite outgrowth is most likely mediated by a combination of cell surface molecules (such as L1, NCAM, integrins) and second messenger systems (such as calcium or phosphorylation) (Schuch et al., 1989; Bixby and Jhabvala, 1990; Doherty et al., 1991; Williams et al., 1994; Doherty and Walsh, 1994; Bixby et al., 1994). The second messenger cascades in turn result in the cytoskeletal changes associated with neurite extension. It has been suggested that changes in phosphorylation states of actin and tubulin may mediate cytoskeletal dynamics. This leads to the following model: in a non-growing neurite, constitutively active receptor PTPases activate non-receptor PTKs. These PTKs in turn phosphorylate actin binding proteins and/or tubulin, which inhibits polymerization. When a growth-promoting extracellular ligand binds to the receptor PTPase, it is inactivated,

resulting in the inactivation of non-receptor PTKs through autoregulation. A decrease in PTK activity would shift the equilibrium towards the non-phosphorylated forms of actin binding proteins and tubulin, which would lead to polymerization and neurite elongation. While highly speculative and certainly overly simplistic, this scenerio provides one useful way to think about how receptor PTPases might function in neurite outgrowth. Aspects of this model are testable by selective inhibition of different points in the biochemical cascade and assaying for the downstream effects.

FUTURE DIRECTIONS

Since the purification of the first PTPase in 1988, rapid progress in this field has placed PTPases in the center of several cellular processes. Nonetheless, several questions remain open. While motivated by the axon-specific patterns of expression in Drosophila, studies of PTPases (except for STEP and PTPH1) have not so far demonstrated similar cell-type or pathway specific expression in the mammalian CNS. One possibility is that there are other, as yet unidentified, PTPase genes that are expressed in more restricted patterns. Another possibility is that different isoforms of the genes already identified may have restricted distributions. We have reviewed here some of the evidence for the production of diverse protein isoforms from a single PTPase gene, either by RNA processing or by post-translational modifications such as glycosylation and proteolytic cleavage. One exciting line of study will be the identification of the isoforms and the generation of specific probes with which to examine their distribution and temporal regulation. Antibodies raised to conserved domains of a PTPase can help identify a family of protein products that may have extracellular domains which differ in peptide sequences and/or glycosylation patterns. On the other hand, isoform-specific antibodies will be

key to investigating differential localization and molecular associations. These kinds of reagents will be useful in studies of the regulation of gene expression, both in vivo and in vitro.

Describing the regulation of diverse isoforms at the mRNA and protein level may provide clues to their cellular functions. However, to understand the biochemical cascades in which PTPases participate will require an understanding of their molecular interactions. Similar to that underway for CD45, the extra- and intra-cellular interactions for each PTPase need to be examined. So far, putative ligands have only been identified for CD45, PTPμ, PTPκ and PTPζ. However, ligand binding does not appear to alter the enzymatic activity of these PTPases. This is in striking contrast to the PTKs, the activity of which is regulated by either heterophilic (eg. trk) or homophilic (eg. Dtrk) binding. In order to find the extracellular ligands for receptor PTPases, expression screening studies may be helpful (Seed and Aruffo, 1987; Aruffo and Seed, 1987). It is important to note that such studies exploring ligands for CD45 have failed probably because ligand binding is strictly dependent on the glycosylation patterns (Trowbridge and Thomas, 1994). Without the identification of such ligands, the regulation of signal transduction by receptor PTPases is doomed to remain a black box. On the other side of the membrane, the cytoplasmic interactions of the PTPases may be amenable to analysis by the yeast two hybrid systems (Fearon et al., 1992). However, because phosphorylation reactions may not require high affinity binding between the PTPase domain and its substrates, functional assays may provide a more direct, yet laborious, approach.

Another promising approach to understanding the functions of receptor PTPases is to look for alterations in specific cellular processes in the absence of PTPase expression. One straightforward approach is to use antisense

oligonucleotides or cDNAs to decrease the expression of a particular PTPase in cultured cells. A more elaborate approach would be to introduce antisense cDNAs in vivo by retroviruses (Galileo et al., 1992). Finally, generation of null mutations in transgenic mice or in Drosophila should provide significant information about the function of specific PTPases. To date, there is no report of PTPase mutants in Drosophila. In mouse, only one isoform of CD45 has been knocked-out. Instead of completely turning off the synthesis of a protein, one can knock out a protein of interest in a specific subcellular location using a combination of intracellular antibodies and laser activation (Dan Jay ref). This approach may be particularly advantageous to analyze processes such as neurite outgrowth and neuronal migration where different regions of the cell may perform different functions. Interpretation of all these experiments can be complicated by the fact that there may be redundant pathways that might mask subtle deficits. Thus, challenging the cells that lack a certain PTPase to a range of conditions in vitro may be required to uncover their functions (Ignelzi et al., 1994).

The abundance and diversity of PTPase genes expressed in the brain provides compelling evidence that PTPases are important players in the complex cell-cell interactions that regulate cell fate determination in the CNS. Because their substrates and extracellular ligands have not yet been identified, the cellular functions of PTPases have been difficult to elucidate. Potentially, each receptor PTPase may lead to the identification of still unknown cell adhesion molecules and novel signal transduction pathways. Therefore, from both the cell biological and neurobiological points of view, the study of receptor PTPases holds the promise of a wealth of information in the future.

TABLE 1
PROPERTIES AND EXPRESSION PATTERNS OF SIX RECEPTOR PTPases EXPRESSED IN THE DEVELOPING RAT BRAIN

PTPase	LAR	CPTP1	PTPδ	PTPζ	RPTPγ	LRP
chromosomal location	human chr. 1	mouse chr. 17	mouse chr. 4	human chr. 7	human chr. 3	mouse chr. 2
major mRNAs (kb)	8.0 6.0 4.0	8.0 6.5	7.0*	9.5 8.5 6.4	9.0 7.0	3.5
E17 CNS						
cortex	+	+	NA	NA**	+	+
midbrain	+	+	NA	NA	+	+
spinal cord	+	+	NA	NA	+	+
P5 CNS						
cortex (I-III)	+	+	NA	NA**	+	+
cortex (IV-VI)	+	+	NA	NA	-	+
subventric. z.	+	+	NA	NA	-	-
hippocamp.	+	+	NA	NA	+	+
cereb (EGL)	+	+	NA	NA	+	-
cereb (IGL)	+	+	NA	NA	+	+
Adult						
brain	+	+	+	+	+	+
lung	+	-	-	-	+	+
kidney	-	-	+	-	+	+
thymus	+	-	-	-	-	-
spleen	-	-	-	-	-	-
testis	+	-	NA	-	-	-

* Three species of mRNA all about 7.0 kb.

** See text for a discussion of PTPζ expression in the developing brain.
NA= data not available.

TABLE 2
PROPERTIES OF SIX NON-RECEPTOR PTPases EXPRESSED IN THE BRAIN

PTPase	PTP-1	STEP	SHP	Syp	P19-PTP	PTPH1
other names	PTP1B	-	PTP1C HCP SH-PTP1	PTPase L1 SH-PTP2 SH-PTP3 PTP1D PTP2C	PTPG1 PTP-PEST	-
chromosomal location	human chr. 20	-	human chr. 12; mouse chr. 6	-	human chr. 7	human chr. 9
mRNA size	3.5	3.0	2.6	6.0	3.5	4.3
alternative splicing	yes	yes	yes	yes	no	no
subcellular location	? ER	cytosolic/ membr.	cytosolic	cytosolic	? nuclear	sub-membr.
enriched in	hippo-campus	striatum	microglia	dev. CNS	dev. CNS	thalamus

Figure 1. Comparison of receptor PTPases expressed in the developing CNS. Six receptor PTPases and their major alternatively spliced isoforms are shown schematically. The immunoglobulin (Ig)-like and fibronectin type III (FN III)-like, carbonic anhydrase (CA)-like, and the phosphatase (PTPase) domains are represented specifically while the unique sequences of each protein is shown with a thick vertical line. The horizontal line crossing all the proteins represents the cellular membrane. For further details about each isoform and the respective references, please see the text.

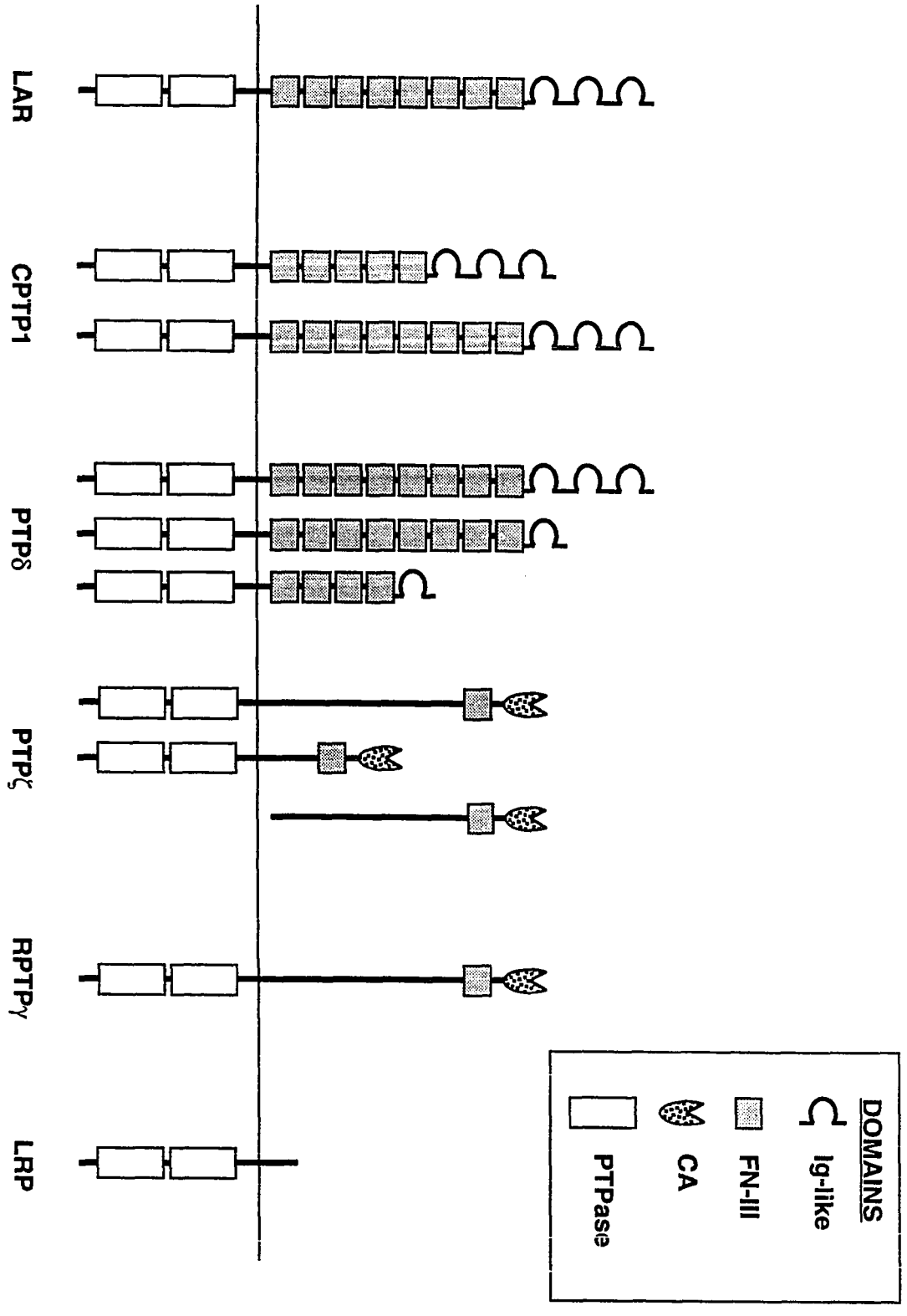


Figure 2. Comparison of non-receptor PTPases expressed in the brain. Six non-receptor PTPases are shown in schematic form. The SH2, PEST- sequence containing, cytoskeleton-associated (ezrin)-like and PTPase domains are represented specifically. Sequences that have no homology to other cDNAs in the database are shown with a thick vertical line. The horizontal line above the six proteins represents the plasma membrane. Some of the PTPases may be associated with the plasma membrane or the ER. For further details and references, please see the text.

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